PLANT PHYSIOLOGY

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ERRATA, VOLUME 3

Page 30, citation 16, following name, insert Über die Verteilung des osmotischen.

Page 36, line 12, for $.4HO_2O$ read $.4H_2O$.

Page 41, last line, for fraction read fractionation.

Page 62, first line of footnote, for $CaCo_3$ read $CaCO_3$.

Page 79, text lines 16 and 18, for xanthroproteic read xanthoproteic.

Page 113, line 7, for homogenity read homogeneity.

Page 169, line 5, for heighth read height.

Page 174, line 13, for flash read flask.

Page 183, line 8, for landwitschaftlichen read landwirtschaftlichen.

Front cover, July no., title 5, for hardness read hardiness.

Page 239, control solution, for $Al_2(So_4)_3$ and $CuSo_4$, read $Al_2(SO_4)_3$ and $CuSO_4$.

Page 309, text line 7, for findinge read findings.

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PLANT PHYSIOLOGY

JANUARY, 1928

STUDIES UPON WHEAT GROWN UNDER CONSTANT CONDITIONS—I

H. L. VAN DE SANDE-BAKHUYZEN

Introduction

From an economic point of view it is very important to find a method of making a trustworthy forecast of the yield of a crop. It has been found that in several agricultural crop plants there is a marked correlation between cool, rainy weather at about the time of flowering and a high crop yield. It has, moreover, been found advantageous to irrigate cereals at this period. The purpose of the present investigation is to determine whether there is a physiological basis for the contention that the flowering stage in plants, especially in wheat, is a critical period in the life cycle. As wheat is one of the most important agricultural plants, in which, moreover, the Food Research Institute of Stanford University has been especially interested, our experiments were carried out with this plant. An early-maturing spring wheat variety, Hard Federation, was chosen. The seed was obtained through the kindness of Dr. V. H. FLORELL, of the U. S. Department of Agriculture, from the Agricultural Experiment Station at Davis, California, and was grown from a pure line.

By the term critical period is meant a period in the life cycle of the plant during which the correlation between external conditions, *i.e.*, rainfall or temperature, and the final yield of the crop is highest. Upon the internal conditions of the plant, *i.e.*, the physiological state of its cells, external conditions exert their influence, thereby changing the physiological activities of the cells, *i.e.*, the development of the grain. We should, therefore, study the internal conditions of the plant throughout its life cycle and, especially, during the period of flowering. If it were possible to detect one weak link in the chain of internal physiological processes accompanying the transition from the vegetative to the reproductive stage (development of

new sporophyte), it would be probable that such a link might act as a limiting factor if the external conditions were such as to attenuate it. In order to study the internal processes which normally go on in a plant it is necessary to grow plants under constant and optimal conditions.

In recent years several investigators have grown plants under constant controlled conditions, but very little is known as yet concerning the internal processes throughout the life cycle. For several reasons the importance of external conditions upon the vital phenomena in plants has been overemphasized (**2**) and even now the effect of one set of constant conditions is usually compared with the effect of another without thoroughly studying the behavior of the plant under any given set of conditions. After this behavior is known exactly, the study of the processes at some other level may be begun, and thus the effect of external conditions ascertained. In our experiments the temperature, illumination, humidity of the air, moisture and salt content of the substratum (sand or solution) were kept as constant as possible.

Experiment room and material

The experiments were carried out in the basement of the Botany Building, Stanford University, in a room 7.8 m. long, 2 m. wide and 2.5 m. high. From the ceiling were suspended fifteen 300-watt Mazda C. lamps, evenly distributed throughout the length of the room. Directly under the lamps, fifteen chimneys, 12 cm. in diameter, connected with a tapering air tunnel about 50 x 25 cm. in diameter, placed under a table, protruded through fifteen holes in the table. This tunnel (of which the end within the room was closed) opened into a side room connected with a long corridor, where a large air space was available. In the middle of one of the long walls opening into the corridor, an electric fan, 50 cm. in diameter, sucked the air out of the closed experiment room. The fifteen chimneys afforded the only entrance for fresh air and a current was produced at the rate of 270 meters per minute, which cooled the lamps and gave the necessary ventilation. This amount of air replacement was required in order to maintain a proper temperature, as the lamps produced much heat. The chimneys ended approximately at the height of the top of the crocks in which the plants were grown. The lamps were 1.20 m. above the top of the crocks.

Two Draper thermographs and one Draper hygrograph recorded the temperature and humidity of the air. The latter was checked with a Tycos dry-and-wet-bulb thermometer. From the average A. M. and P. M. readings an average daily reading was found; from these data the mean daily temperature and humidity with probable errors were calculated. Daily fluctuations in the temperature did not occur; the humidity of the air showed only secular fluctuations. If the latter showed a trend to rise

or fall, the values between the end and the beginning of the week did not differ more than 5 per cent. The largest deviation from the mean temperature amounted to $\pm 1.2°$ C., the largest deviation from the mean humidity was ± 8 per cent. Both maximum deviations were rare, as can be seen from the probable errors, given in the next section. No special devices were used for keeping temperature and humidity of the air constant, as during the experiments the air in the long corridor was very constant in these respects.

During the entire period of the experiments the lights burned 24 hours a day. In order to economize light energy, white cardboard screens suspended from the ceiling surrounded the table; in this way reflection took place in all directions. If the distance between the lamps and the plants is large enough, the light intensity must remain constant independent of the distance. In our case it decreased somewhat less than the first power of the distance. Fifteen lamps of 300 watts each produce $15 \times \frac{4900}{2} = 36750$ lumens. The area between the screens was $25 \times 2.1 = 52.5$ sq. ft., *i.e.* per sq. ft. a quantity of $\frac{36750}{52.5} = 700$ lumens or foot-candles was received. As the lamps were, however, so close to the plants that the reflectors intercepted part of the light, the intensity as measured with a Leeds and Northrup Macbeth Illuminometer gave only a value of 600 foot-candles. After about a month the lamps had lost 20 per cent. of their intensity and were renewed.

In the first experiment the sand in which the wheat was grown was washed with 3 per cent. hydrochloric acid, then with water to neutrality and finally with distilled water. In the second experiment, however, this procedure was omitted as it was too laborious and as the test for chlorine showed that only a very slight amount was present. A three-salt solution with an osmotic pressure of 1 atmosphere was used. Iron was added in the form of tartrate. By weighing the crocks, a soil moisture of about 10 per cent. was maintained throughout the experiment. In the beginning of the experiment every other day, later on every day, the water lost by evaporation and transpiration was restored in the form of distilled water or nutrient solution.

After a selection of the seed by hand and by a screen, all the seeds above 50 mg. and below 40 mg. were discarded. The seeds were laid out to germinate on gauze, stretched over hollow glass rings floating in water, a method used in the Botanical Laboratory of Utrecht (Holland) and giving very uniform germination. After the roots and coleoptiles had developed for a few days, a third selection was carried out. In each of the 50 two-gallon crocks six seedlings were planted; the crocks were placed in two rows of 25 along the length of the table.

The plants were supported by protected wires (pipe-cleaners) fastened to a stick placed in the center of the sand. Each plant formed 5 leaves. The tillers began to develop very late in the life cycle but were suppressed as soon as they became visible. In this way standard plants were obtained with 1 stem and 5 leaves.[1]

For every harvest an average crock was selected. In order to study the phenomena of growth in greater detail the fresh weight and dry weight of the stems + leaves and of the ears were determined separately. In the second experiment the fresh weight and dry weight of the leaves were determined as well; they were cut off along the pulvinus above the ligula. The fresh and dry weights were determined by weighing in bottles to 0.1 mg. (a) immediately after cutting and (b) after drying to constant weight at 105° C. in an oven. The outline of every leaf was traced on paper and the leaf area determined in the first experiment by weighing, in the second with a planimeter. The remaining part of the stem and sheaths is designated hereafter as "stems alone." The length of the different organs of a number of plants was measured daily in order to get an idea of the daily growth in length. Transpiration determinations with sand and water cultures were also carried out.

The number of plants harvested was 5[1] or 6, in all but two of the crocks (11 and 11R 1926) in which it was only 2. The probable errors were calculated as $r = \pm 0.6745\sqrt{\frac{\Sigma(\bar{x}-x)^2}{n(n-1)}}$. Though the number of individuals is too small for the calculation of significant probable errors, their values give a fair idea of the range of variability of the data.

General growth features in the two experiments with sand cultures

The first experiment was carried out in January, February and March, 1925; the second one in September, October and November, 1926.

AVERAGE DAILY TEMPERATURE

	Exp. 1925	Exp. 1926
January	22.1 ± 0.16	26.3 ± 0.08
February	22.9 ± 0.10	

AVERAGE DAILY HUMIDITY OF THE AIR

January	57.5 ± 0.88	58.1 ± 0.38
February	67.6 ± 0.75	

[1] A few plants formed 6 or 4 leaves. These plants were discarded. All the other plants were used for the determination of the mean values, except a few plants of which the total leaf area could not be determined.

The following table gives the approximate day on which the successive organs become visible:

TABLE I

GROWTH OF WHEAT IN SAND CULTURES

PLANT ORGAN	EXPERIMENT 1925 AGE OF PLANT	EXPERIMENT 1926 AGE OF PLANT
	Days	Days
1st leaves	7	5
2d leaves	9–10	7–8
3rd leaves	15	12
4th leaves	19	16
5th leaves	22	19
Ears	32	27–28
Stamens	38–40	± 30–31

In the second experiment, the temperature being 4° C. higher[2] than in the first experiment, the appearance of the successive leaves was about 3 days earlier, the appearance of the ears 4–5 days earlier, that of the stamens about 8–9 days earlier. As the plants in the second experiment reached their final size in 30 against 39 days, they were accordingly shorter (60 against 79 cm.). Every 3.5 days a new leaf appeared and in both experiments the 5th leaves appeared 14 days after the 1st leaves.

The average number of spikelets per ear in the first experiment was about 11, the number of kernels 22; in the second experiment 9 and 12, respectively. The low average number of kernels in the second experiment is probably due to the high temperature, which made several spikelets sterile; the average rate of growth, however, did not lag behind that in the first experiment. In the first experiment the kernels were ripe after 72 days, *i.e.* they showed a per cent. dry weight of 89, which means that they were air dry. The final air dry weight of the kernels developed in this experiment was about 50 mg. for the 1st and 2d spikelets and 40 mg. for the 3rd spikelets, which is a fair weight compared with the original seed (about 45 mg.). Wheat plants of the same variety sown in spring in the Experiment Garden of Stanford University were ripe after 90–100 days and had about 11 spikelets and about 30 seeds.

The first leaf persisted long after flowering. The leaves were somewhat darker green than the leaves of plants grown during spring in the field, the lower leaves were markedly longer.

The first tillers appeared only after one month, *i.e.* the stems began to develop very early; in the second experiment the first node had developed

[2] During the second experiment the temperature in the corridor, *i.e.* of the incoming air, was about 4° C. higher than during the first experiment.

on the 17th day. The third leaf is inserted at this node, the fifth and upper leaf at the third node.

The starch grains of the kernels, developed in these experiments, did not show lamellation at room temperature or after heating in water to 50° C. or higher, as has been shown by the writer (1). The ears of plants grown under the constant conditions did not show the typical brown color of Hard Federation, but were quite white. Plants grown from this seed in the field did not show a difference from plants grown from the original seed.

I wish to express my hearty thanks to the Directors of the Food Research Institute for the opportunity they gave me to carry out these experiments and to Dr. G. J. PEIRCE, who kindly provided me with working facilities in the Botany Building.

FOOD RESEARCH INSTITUTE AND DEPARTMENT OF BIOLOGY,
STANFORD UNIVERSITY.

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STUDIES UPON WHEAT GROWN UNDER CONSTANT CONDITIONS—II

H. L. VAN DE SANDE-BAKHUYZEN

(WITH FIVE FIGURES)

The water content of different organs throughout the life cycle

Usually the water content of an organ is given as a percentage of the dry weight or of the fresh weight. In this paper per cent. dry weight is defined as $\frac{\text{dry weight}}{\text{fresh weight}}$, and per cent. moisture as $\frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}}$ the sum of both is 100 per cent. If the increase in moisture (fresh weight minus dry weight) goes parallel with the increase in dry weight, both quotients remain the same; if the dry weight increases more rapidly than the moisture, or if water is lost, the per cent. dry weight increases, and the per cent. moisture decreases.

The stem and leaves

As fig. 1 shows, in both experiments (1925 and 1926) the percentage dry weight of the stems and leaves (ears excluded) goes up rather smoothly until the time of flowering. In experiment 1925 the plants harvested on the 41st day were the first that had flowered, flowering having occurred between the 38th and 40th days. In experiment 1926, five of the six plants harvested on the 31st day (crock 43) had not flowered and showed a dry weight of 19.5 per cent.; one plant had flowered and showed a value of 24.2. All the plants harvested after the 39th day in experiment 1925 and after the 31st day in 1926 had flowered.

The curves show evidently that there is a sudden increase in the percentage of dry weight of the stems and leaves after flowering. In 1925 this sudden increase was observed 1–2 days after flowering, in 1926 about 2–3 days after flowering (33rd day) or one day for the single plant which flowered on the 31st day.

The phenomenon that the dry weight per cent. increases or that the moisture per cent. decreases at the time of flowering does not, however, show whether this is due to a sudden increase in dry weight alone, or to an increase both in dry weight and moisture, the latter increasing less rapidly than the former, or to a sudden loss of water whether or not accompanied by an increase or decrease in dry weight. The only way to find the real cause of this change in moisture per cent. is to consider the absolute dry weight and moisture. We find then that the moisture of the stems and leaves increases until the time of flowering but decreases markedly just after flowering.

In order to make the plants harvested on successive days more comparable, an effort was made to eliminate sampling errors. In an ideal experiment no such sampling errors would have occurred, *i.e.*, all the first, second, etc., leaves would have had the same leaf area, so that the total leaf area on the same day would be the same for all plants. If we assume that after the leaves were full-grown the chemical efficiency (**2**) (assimilatory

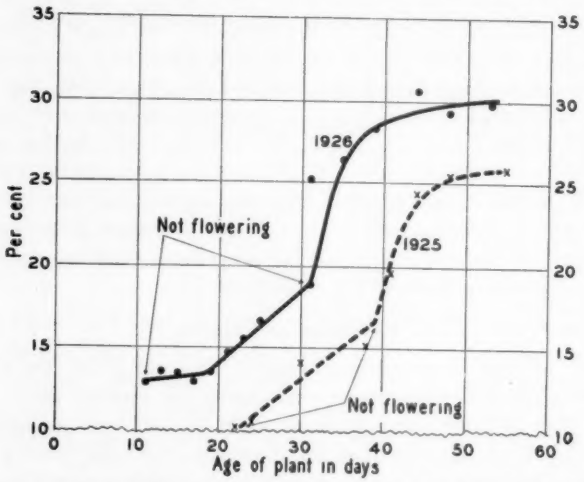

FIG. 1. Percentage dry weight of stems and leaves in the experiments of 1925 and 1926. On the 31st day of the latter experiment one of the six plants had flowered.

capacity) of the leaves and also the structural efficiency (**2**) (leaf area/total dry weight) were the same in all the plants, they would all reach the same final length. In this case we could compare the moisture of the plants in all the crocks on successive days without further calculations. In our case, however, the height and the leaf area were not the same in all plants, so that we must compare the plants harvested with an average plant. Without going into further details it is evident that the dry weight at any time depends upon the leaf area and that the correlation between both values must be 100 per cent. if both efficiencies are the same in all plants.[1] Therefore, we can to a large extent eliminate the sampling errors for the date on which all the leaves are full grown by dividing the moisture and dry weight by the leaf area.[2] The probable error of the mean values calculated

[1] In this case the leaf area and the dry weight on any day depend only upon the weight of the original seed.

[2] That we improve our data by dividing the moisture or dry weight by the total leaf area or stem length is shown by the fact that the variation coefficient of the data calculated in this way is much less than that of the original data.

TABLE II

MOISTURE AND DRY WEIGHT IN MG. OF STEMS AND LEAVES BEFORE AND AFTER FLOWERING. EXPERIMENT 1925

CROCK NO.	AGE	STEM LENGTH	MOISTURE / LENGTH	DRY WEIGHT / LENGTH	LEAF AREA	MOISTURE / LEAF AREA	DRY WEIGHT / LEAF AREA
	Days	cm.			cm^2.		
45	38	55.2	60.7 ± 2.06	10.9 ± 0.30	62.2	53.8 ± 2.64	8.9 ± 0.50
Flowering 43	41	77.8	43.1 ± 1.01	10.7 ± 0.13	—	—	—
07	44	77.1	37.2 ± 0.83	12.0 ± 0.28	66.6	42.0 ± 1.35	13.6 ± 0.72
23	48	78.4	41.2 ± 1.51	13.8 ± 0.48	74.1	43.6 ± 2.02	14.7 ± 0.68
33	55	80.6	38.7 ± 0.58	13.5 ± 0.33	74.1	42.0 ± 0.98	14.6 ± 0.42

from the separate data of the plants in one crock may give us an idea whether this correlation is far off from 100 per cent. or not. The moisture of such a plant with unit leaf area increases until the time of flowering and then falls off suddenly, as we shall see in tables II and III. It is, however,

TABLE III

MOISTURE AND DRY WEIGHT IN MG. OF STEMS AND LEAVES BEFORE AND AFTER FLOWERING. EXPERIMENT 1926

CROCK NO.	AGE	STEM LENGTH	MOISTURE / LENGTH	DRY WEIGHT / LENGTH	LEAF AREA	MOISTURE / LEAF AREA	DRY WEIGHT / LEAF AREA
	Days	cm.			cm.²		
14	23	37.2	48.9±2.08	9.0±0.41	56.0	32.4±0.58	6.0±0.42
39	25	42.3	—	9.2	55.9	—	6.9±0.16
43	31	59.6	45.5±1.51	10.6±0.36	65.3	41.6±0.47	9.7±0.24
Flowering	31	(61.1)	(32.4)	(11.0)	(51.7)	(38.3)	(12.9)
11	33	58.3	40.0±0.61	11.9±0.07	—	—	—
09	35	64.5	32.7±0.53	11.7±0.15	58.5	36.0±0.36	12.9±0.42
11R	39	60.7	31.7±1.02	12.6±0.05	55.8	34.5±0.71	13.7±0.24
30	44	61.1	29.4±0.68	12.9±0.38	53.9	33.4±0.60	14.7±0.32
10	48	59.2[a]	31.9±0.87[a]	12.5±0.76[a]	60.0[b]	33.6±0.51[b]	14.3±0.24[b]
41	53	60.1	31.3±1.39	13.2±0.74	55.7	33.6±0.67	14.7±0.40

[a] Average of 4 plants.

[b] Average of 2 plants, as the total leaf area of 2 plants could not be determined.

impossible to find in this way the moisture maximum, which occurs just before flowering, as the plants harvested on the 38th day (experiment 1925) and the five non-flowering plants on the 31st day (experiment 1926) were not to flower till 1–2 days later. This is shown by a comparison of stem length (ear excluded) and leaf area. In 1926 for all the plants harvested after flowering (21 plants) the ratio stem length/leaf area is 1.06 ± 0.015, while for the five non-flowering plants in crock 43 it is 0.92 ± 0.03, proof that the stems in the latter crocks are not yet full grown. We come to the same result if we compare the lengths of the successive internodes. As is generally known the length of the n^{th} node is about the arithmetic mean of the $(n-1)^{st}$ and $(n+1)^{st}$ internode. If we calculate the ratio of the length of the (2d + 4th [ear excluded]) internode and twice the length of the 3rd internode, we find in 1926 for the flowering plants an average of 1.08, while for the five non-flowering plants on the 31st day it is 1.00. In 1925 the latter values for the flowering plants are 1.09, for crock 45 (38th day), which has not yet flowered, 0.69. Therefore, if there is a loss

of water at the time of flowering, it will be larger than is found by dividing the moisture by the leaf area, as the maximum moisture found is less than the real maximum moisture.

If we divide the moisture by the stem length, we assume that the massive cylinder of tissue in the stem around the central cavity is of uniform radius and thickness in all plants and in all internodes; the last internode, however, is much thinner than the lower ones. Therefore in full-grown plants the average moisture and dry weight per cm. stem length must be smaller than in plants in which the last internode is not yet full grown. Moreover, the moisture per cent. decreases in the successive internodes. Thus we find too high a value for the loss of water.

We may expect, therefore, that the true loss of water lies between the value for the loss found by dividing the moisture by the leaf area and the value found by dividing it by the stem length. In tables II and III both sets of values are given. In crock 14 (experiment 1926), harvested on the 23rd day, all the leaves were full grown, but the ears were not yet formed, so that the moisture and the dry weight of the entire tops were divided by the leaf area or the length of the plant from the base up to the 5th (last) ligula. The latter value has, therefore, only a relative significance, as it is not quite comparable with the values of the plants in which the stems were nearly or quite full grown.

Tables II and III show that in both experiments 1925 and 1926 there occurs a sudden loss of water within 1–2 days after the time of flowering. In experiment 1925 the moisture of stems + leaves calculated per cm. stem length dropped after flowering from 60.7 ± 2.06 mg. (38th day) to 43.1 ± 1.01 mg. (41st day), which means a loss of 29 per cent. of the moisture present on the 38th day. The moisture calculated per cm^2. leaf area drops from 53.8 ± 2.64 mg. (38th day) to 42.0 ± 1.35 (41st day), which equals a loss of 22 per cent. The true loss lies between 22 and 29 per cent. After the 41st day, *i.e.* 1–2 days after flowering, the values of the moisture decrease slightly or remain practically constant until the 55th day.

The same result was obtained in experiment 1926, as is shown in table III. In the five non-flowering plants of crock 43 (31st day) the moisture per cm. stem length is 45.5 ± 1.51 mg., which is in fair agreement with the value of crock 14 (23rd day) : 48.9 ± 2.08 mg. The one flowering plant in crock 43 (the values for this plant are given in parentheses) shows a moisture per cm. stem length of 32.4 mg. which agrees closely with the value of crock 09 (35th day). The values of crock 11 (2 plants only) shows an intermediate position between crocks 43 and 09. The value of crock 09 shows a loss of 27 per cent. compared with the moisture before flowering. After the 35th day the moisture decreases slightly until the 53rd day. The moisture calculated per $cm.^2$ leaf area increases from the 23rd day

(32.4 ± 0.58 mg.) until the time of flowering (41.6 ± 0.47 mg.) and decreases 1–5 days after flowering (38.3 mg. for the one flowering plant in crock 43 and 36.0 ± 0.36 mg. in crock 09). The loss of water in crock 09, calculated in this way, is 13.5 per cent. of the moisture present in the five plants of crock 43. There is still a further decrease between the 35th and 44th day and then the value remains constant until the 53rd day. The true loss of water between the 31st day and the 35th day lies between 13.5 and 27 per cent. The dry weight of stems and leaves increases all the time. In experiment 1926 the mean value of the dry weight on the 33rd day was probably found too high, moreover the plants (only 2) were very short. Therefore the moisture on this day also may be too high (40.0 ± 0.61), which is the more probable if we compare this value with the values of the single flowering plant in crock 43 and of the plants in crock 09.

In experiment 1926 the probable errors of the moisture and dry weight per unit stem length are 3 per cent. of the total value; if calculated per unit leaf area they are 2.2 per cent. In experiment 1925 these values are respectively 2.6 and 4.2 per cent. The latter value in 1925 (4.2) is higher than in 1926 (2.2), because in 1925 the leaf area was determined by weighing paper tracings, which gave less accurate results than the determination with a planimeter in 1926. It seems, therefore, that the best results are obtained by dividing the total moisture or dry weight of stems + leaves by the leaf area. This corresponds with the fact that the probable errors of the values per unit leaf area for the "leaves alone" are very low, as will be evident later.

The "stems alone"

For the values of the percentage of dry weight of the stems alone (stems + sheaths) the same holds as for the stems + leaves; this is shown in fig. 2, and in table IV. The single flowering plant in crock 43 had a much higher dry weight per cent. (27.5) than the five non-flowering plants (18.9). Crock 09, harvested 4 days later, shows a value of 28.1. It is evident that there is a sudden rise in the values just after flowering.

Whether in the stems alone the absolute weights of moisture and dry weight, or the weights per unit stem length or unit leaf area be employed, exactly the same results are obtained as when these calculations are made for the stems + leaves. The values for the moisture per cm. stem length on the 23rd and the 27th days agree fairly well with the value of the five non-flowering plants on the 31st day, being respectively 30.4 ±1.11, 29.3 ± 2.11 and 31.7 ± 1.12 mg. The values on the 27th day are too low, and indicate not merely for the stems but also for the leaves an abnormally low moisture and dry weight. This is probably due to the fact that this crock had accidentally a soil moisture which was much too low; the values for the plants

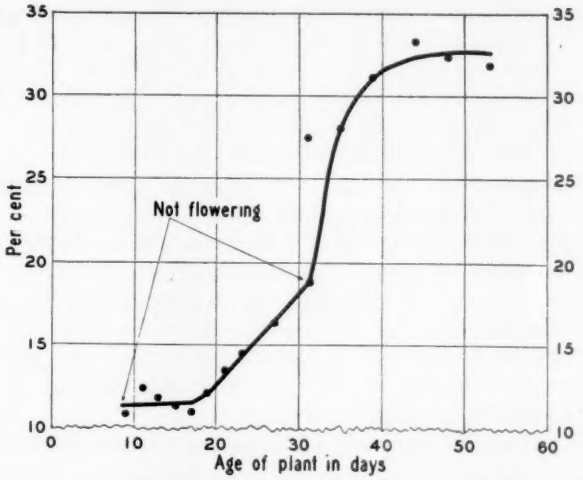

FIG. 2. Percentage dry weight of stems alone. Experiment of 1926. On the 31st day one of the six plants had flowered.

were not very uniform and this is reflected in a very high probable error. The one flowering plant on the 31st day shows a value (22.7 mg.) which agrees closely with the value on the 35th day (22.4 ± 0.38 mg.). After the 39th day the moisture and the dry weight remain constant until the 53rd day.

TABLE IV

MOISTURE AND DRY WEIGHT IN MG. OF STEMS ALONE BEFORE AND AFTER FLOWERING. EXPERIMENT 1926

CROCK NO.	AGE	STEM LENGTH	MOISTURE / LENGTH	DRY WEIGHT / LENGTH	LEAF AREA	MOISTURE / LEAF AREA	DRY WEIGHT / LEAF AREA
	Days	cm.			cm.²		
14	23	37.2	30.4±1.11	5.1±0.21	56.0	20.2±0.54	3.4±0.11
39	25	42.3	—	5.7	55.9	—	4.1±0.11
42	27	42.5	29.3±2.11	5.7±0.33	—	—	—
43	31	59.6	31.7±1.12	7.4±0.29	65.3	29.0±0.40	6.8±0.24
Flowering		(61.1)	(22.7)	(8.6)	(51.7)	(26.8)	(10.1)
09	35	64.5	22.4±0.38	9.0±0.20	58.5	24.7±0.35	9.8±0.32
11R	39	60.7	21.5±0.78	9.8±0.07	55.8	23.4±0.54	10.7±0.24
30	44	61.1	20.1±0.38	10.1±0.33	53.9	23.3±0.67	11.5±0.30
10	48	59.2[a]	21.5±0.61[a]	10.0±0.43[a]	60.0[b]	22.7±0.59[b]	11.1±0.24[b]
41	53	60.1	21.7±0.98	10.1±0.54	55.7	23.4±0.49	10.9±0.28

[a] Average of 4 plants.

[b] Average of 2 plants; see table III.

The same holds for the moisture and dry weight calculated per unit leaf area. For the reasons above given, the loss of water calculated in this way is smaller than when calculated per unit stem length, the loss on the 35th day (crock 09) being respectively 15 per cent. and 30 per cent. of the moisture present in the five non-flowering plants on the 31st day. The true loss up to the 35th day lies between 15 and 30 per cent.

The dry weight per unit leaf area increases more than does the dry weight per unit stem length as the leaf area remains constant after the 23rd day, while the dry weight of the stem increases proportionately more with its length, though the dry weight per unit length also increases.

If in the "stems alone" the values of fresh weight and dry weight are calculated per unit leaf area, the probable errors are absolutely and relatively smaller (respectively 2.1 per cent. and 2.7 per cent.) than if calculated per unit stem length (respectively 3.5 per cent. and 3.4 per cent.), as was also the case for the stems + leaves.

For the plants in experiment 1925 no data are available for the stems alone.

The ears

There is some evidence that in the ears there is also a sudden loss of water at the time of flowering. This is indicated in both experiments not only by a rapid increase in the percentage of dry weight between 1–2 days before flowering and 2 days after flowering, but we find this loss also, if we consider the absolute values of moisture and dry weight.

The same results are obtained if the moisture per unit ear length or per unit leaf area be calculated. In the experiment of 1925 the moisture increases again 4–5 days after flowering; in that of 1926 it increases 5 days after flowering, so that on the 39th day the original moisture is exceeded again. The data are given in table V.

TABLE V

MOISTURE AND DRY WEIGHT OF EARS IN GM. BEFORE AND AFTER FLOWERING

CROCK NO.	EXPERIMENT 1925				EXPERIMENT 1926				
	AGE	MOISTURE	DRY WEIGHT	NO. OF SPIKELETS	CROCK NO.	AGE	MOISTURE	DRY WEIGHT	NO. OF SPIKELETS
Not flowering .. 45	Days 38	gm. 0.519	gm. 0.130	10.5	43	Days 31	gm. 0.5910	gm. 0.1722	10.4
Flowering 43	41	0.431	0.193	10.6	43 II[a]	31	0.4508	0.1495	9.0
07	44	0.511	0.267	11.0	11	33	0.4852	0.1738	10.5
					09	35	0.4961	0.2207	9.3
					11 R	39	0.5967	0.2860	9.0

[a] 43 II means plant II of crock no. 43.

The leaves

The percentage of moisture of the leaves decreases as we ascend from the lower to the upper leaves, *i.e.*, the latter have relatively more dry weight and less moisture than the former. Table VI shows the differences in dry weight per cent. in the successive leaves harvested on different days (experiment 1926).

TABLE VI

PERCENTAGE DRY WEIGHT OF LEAVES EXPERIMENT 1926

AGE	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES
Days	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
23	15.1	14.6	16.0	18.0	20.2
35	18.0	17.1	19.3	22.0	24.7
53	24.4	21.2	21.7	24.4	27.0

This table shows that though the first two leaves show about the same per cent. dry weight, this value increases from the 2d leaf to the 5th. On the 53rd day the value for the 1st leaf is much higher than that of the 2d, because the former is yellowing and gradually dying off. For the other days and in experiment 1925 this difference in percentage of dry weight in the successive leaves is just as striking. Table VII gives these values for the upper and lower leaves of tomatoes (table XXIV, KRAUS and KRAYBILL (7)) and of leaves of corn (SCHWEITZER (11)).

TABLE VII

PERCENTAGE DRY WEIGHT OF LEAVES OF TOMATO AND CORN QUOTED FROM KRAUS AND KRAYBILL AND SCHWEITZER

PLANT	LOWER LEAVES	UPPER LEAVES
	Per cent.	Per cent.
Tomatoes	14.00	18.60
Corn (68 days old)	12.30	14.13

Fig. 3 shows the percentage dry weight of the 3rd leaves harvested on successive days (experiment 1926). The value increases approximately rectilinearly until the time of flowering. The one flowering plant in crock 43 shows a much higher value than the five non-flowering plants.

It is evident that the value on the 35th day is much higher than could be expected from the trend before flowering. After the 35th day the trend is approximately the same as before flowering, only at a higher level. The same holds for the other leaves. Again the dry weight and moisture per cm.² of leaf area must be calculated in order to eliminate sampling errors. Usually only the percentage of moisture or percentage of dry weight is given in the literature. JOST (3) quotes a case where erroneous results are obtained if the morning and evening N-content of leaves is calculated per unit leaf dry weight, because a unit leaf area weighs less in the morning than it did the previous evening. Moreover, in calculating the apparent

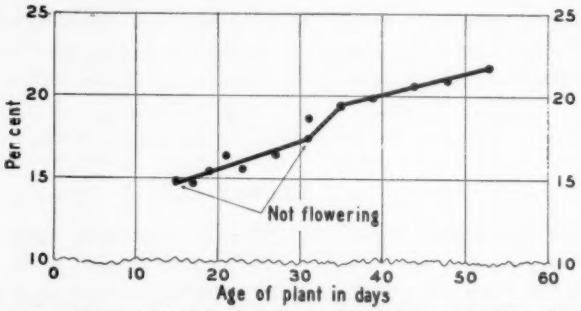

FIG. 3. Percentage dry weight of the 3rd leaves. Experiment of 1926. On the 31st day one of the six plants flowered.

assimilation of an entire plant it is usually advantageous to calculate the daily increase in dry weight per unit leaf area instead of per unit leaf dry weight. Though we are quite aware that it is much more laborious to calculate the moisture by the former method than by the latter, we think this latter method should be employed to a much greater extent than it has been heretofore.

In evaluating the moisture or dry weight per cm.² of leaf area, the order of magnitude of the probable error to be expected must first be considered. In our second experiment the fresh weight and dry weight were determined with an error of about 0.1 mg., *i.e.*, the probable error of the moisture is about 0.1–0.2 mg. The outlines of the leaves were traced on paper. What errors were made in tracing was not determined, though it is probable that in the tracing of the lower leaves a relatively larger error was made than in the tracing of the upper leaves. In the determination of the leaf area with a planimeter an average error of $\pm$ 0.1 cm.² was made; every leaf was measured three times and if the values differed more than $\pm$0.1 cm.², more measurements were made. An error of $\pm$ 0.1 cm.² leaf area affects the dry weight per cm.² in the second decimal of a milligram and the moisture to an extent of $\pm$ 0.1 mg.

If the total dry weight of every leaf be divided by its leaf area, the assumption is made that every leaf has the same thickness and structure, *i.e.*, that there exists a correlation of 100 per cent. between leaf area and dry weight or moisture. Though this correlation has not been calculated, it is reflected in the low values of the probable errors, which are about 1.5 per cent. of the mean values per cm.², which is much less than found in the stems and leaves or stems alone.[3] In a few cases, however, in which the leaf areas are very large or very small there is a slight correlation between the leaf area and the values per cm.², proof that these leaves are different in thickness or structure.

The average dry weight per cm.² of leaf area of all the leaves is 2.5 ± 0.04 mg., the average moisture 11 to 12 ± 0.18 mg. (tables VIII and IX). The largest deviation from the mean moisture very rarely exceeds 0.8–1.0 mg. (except for the single flowering plant of crock 43). In plants harvested on the same day the values of dry weight and moisture can be compared with checks and counter checks as the following relations are known.

1. Leaf area and leaf dry weight (dry weight per cm.² leaf area).
2. Leaf dry weight and fresh weight (percentage of dry weight or percentage of moisture).
3. Leaf area and leaf moisture (moisture per cm.² leaf area).
4. Dry weight of a single leaf and dry weight of adjacent leaves, or dry weight of the entire plant.

The correlation between the dry weight of the 1st leaf and the dry weight of stems or entire tops exceeded in many cases 0.90.

If 1 shows a considerable deviation from the mean (13 per cent. of the 237 cases showed a deviation larger than 0.2 mg.), this is an indication that probably a mistake has been made in tracing the leaf area, as in these cases the moisture per cm.² is also abnormal; the percentage of dry weight may be quite normal. An abnormality in water content is reflected in moisture per cm.² and percentage of moisture.

Aside from these abnormal values, the values are very consistent, as shown not merely by the low probable errors, but also by the mean values for the crocks harvested on successive days, as shown in tables VIII and IX. All the leaves harvested[4] were used for the determination of the mean values and the probable errors. The value for the dry weight of the 5th leaves of crock 41 is certainly too low, as one plant showed a value of 3.1; if we

[3] See also footnote 2, p. 8.

[4] A few 5th leaves were too much curled to allow accurate determination of the leaf area.

omit this value the dry weight would be 4.0 ± 0.14 mg., which is more in agreement with the values on the previous days.

All the leaves of the plants harvested were full grown except the 4th leaves of no. 46 and the 5th leaves of no. 23, which are placed between brackets.

TABLE VIII

LEAF MOISTURE IN MG. PER CM.² LEAF AREA. EXPERIMENT 1926

CROCK NO.	AGE	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES
	Days	mg.	mg.	mg.	mg.	mg.
45	9	13.1 ± 0.35				
26	11	12.6 ± 0.30				
21	13	12.8 ± 0.16	12.2 ± 0.18			
12	15	12.8 ± 0.17	12.3 ± 0.18	11.7 ± 0.11		
37	17	12.9 ± 0.70	12.7 ± 0.20	11.7 ± 0.17		
46	19	14.1 ± 0.39	12.8 ± 0.27	12.1 ± 0.22	[12.1 ± 0.13]	
23	21	14.7 ± 0.34	12.9 ± 0.21	12.2 ± 0.11	11.6 ± 0.15	[12.1 ± 0.46]
14	23	13.8 ± 0.07	13.1 ± 0.28	12.3 ± 0.13	11.8 ± 0.17	11.7 ± 0.28
39	25	—	—	—	—	—
43	31	14.1 ± 0.11	12.9 ± 0.06	12.9 ± 0.23	12.6 ± 0.13	12.0 ± 0.16
		(12.1)	(12.3)	(11.4)	(11.2)	(11.0)
09	35	12.1 ± 0.11	12.5 ± 0.15	11.6 ± 0.19	10.9 ± 0.13	10.4 ± 0.08
11R	39	—	11.4 ± 0.20	11.2 ± 0.37	10.7 ± 0.13	10.3 ± 0.11
30	44	9.6 ± 0.35	11.2 ± 0.08	10.9 ± 0.17	10.7 ± 0.10	9.9 ± 0.17
10	48	10. 9± 0.20	10.9 ± 0.11	10.9 ± 0.14	10.5 ± 0.18	10.4 ± 0.05
41	53	8.5 ± 0.20	10.8 ± 0.12	10.7 ± 0.13	10.6 ± 0.19	10.4 ± 0.07
Average probable error		± 0.21	± 0.17	± 0.18	± 0.15	± 0.13

In crock 43 ( ) means one plant flowering, the five other plants not yet flowering. After the 32–33rd day all the plants have flowered.
[ ] means leaves not yet full grown.

If the tables are read left to right, we find that the dry weight per cm.² increases while the moisture per cm.² decreases. If this be expressed in terms of percentage of dry weight, the values increase from the lower leaves to the upper leaves (table VI). Before flowering, the moisture of second leaves is always lower than the moisture of first leaves, but the dry weight of the former is often equal to or lower than that of the latter. Thus the second leaf often has a lower per cent. dry weight than the first leaf, as found above. The average variation coefficients for the dry weights of the successive leaves are approximately the same; for moisture, the coefficients decrease markedly, because the absolute probable error (or standard deviation) decreases more than the mean values themselves decrease as we ascend from the first to the fifth leaf.

TABLE IX

LEAF DRY WEIGHT IN MG. PER CM.^{2} LEAF AREA. EXPERIMENT 1926

CROCK NO.	AGE	1ST LEAVES	2D LEAVES	3D LEAVES	4TH LEAVES	5TH LEAVES
	Days	mg.	mg.	mg.	mg.	mg.
45	9	1.9 ± 0.03				
26	11	2.0 ± 0.02				
21	13	2.2 ± 0.05	2.2 ± 0.03			
12	15	2.1 ± 0.02	2.2 ± 0.04	2.05 ± 0.02		
37	17	2.1 ± 0.10	2.2 ± 0.03	2.0 ± 0.03		
46	19	2.2 ± 0.06	2.2 ± 0.02	2.2 ± 0.02	[2.3 ± 0.04]	
23	21	2.4 ± 0.02	2.4 ± 0.03	2.3 ± 0.05	2.5 ± 0.04	[2.9 ± 0.05]
14	23	2.3 ± 0.05	2.4 ± 0.02	2.4 ± 0.01	2.6 ± 0.05	3.0 ± 0.07
39	25	2.4 ± 0.01	2.4 ± 0.01	2.6 ± 0.02	2.7 ± 0.05	3.0 ± 0.02
43	31	2.8 ± 0.09	2.5 ± 0.02	2.7 ± 0.02	2.8 ± 0.05	3.4 ± 0.04
		(2.6)	(2.5)	(2.6)	(2.8)	(3.2)
09	35	2.7 ± 0.06	2.6 ± 0.04	2.8 ± 0.01	3.1 ± 0.01	3.5 ± 0.09
11R	39	2.7 ± 0.0	2.7 ± 0.0	2.8 ± 0.05	3.1 ± 0.0	3.5 ± 0.06
30	44	2.7 ± 0.04	2.7 ± 0.02	2.8 ± 0.02	3.4 ± 0.06	4.0 ± 0.08
10	48	2.9 ± 0.04	2.7 ± 0.07	2.9 ± 0.05	3.2 ± 0.07	3.9 ± 0.09
41	53	3.0 ± 0.10	2.9 ± 0.10	3.0 ± 0.06	3.4 ± 0.04	3.8 ± 0.16
Average probable error		± 0.05	± 0.03	± 0.03	± 0.04	± 0.08

In crock 43 ( ) means one plant flowering, the five other plants not yet flowering. After the 32–33rd day all the plants have flowered.
[ ] means leaves not yet full grown.

Reading the table of the dry weights from above downward, we find that the values for all the leaves increase throughout the life cycle.

If we read the table of the moisture from above downward, we see that this value increases for all the leaves up to the time of flowering, the five non-flowering plants in crock 43 showing the highest figures. But the relative increase in moisture is less than the relative increase in dry weight, so that the dry weight per cent. increases from the beginning until the time of flowering, as shown in fig. 3.

The single flowering plant (43 II) in crock 43 (between brackets) shows a considerably lower moisture. That this is not an accidental deviation is shown by the fact that the stems and the ears also had a lower moisture content. This is also shown by the values of crock no. 09, harvested four days later, as shown in table X.

In order to ascertain whether the difference between 43 II and the mean of the five non-flowering plants and also the difference between the five non-flowering plants and the one flowering plant in no. 43 + the six plants in crock 09 (which had flowered) is significant, how many times this differ-

TABLE X

LEAF MOISTURE IN MG. PER CM.[2] LEAF AREA OF PLANTS WHICH HAD PRODUCED STAMENS

CROCK NO.	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES
43 II	12.1	12.3	11.4	11.2	11.0
09	12.1	12.5	11.6	10.9	10.4

ence exceeds the probable error can be calculated. As the number of samples is, however, small, it is preferable to use the method of FISHER (**6**), which determines the probability (1–P) that a difference between both values, larger than the difference observed, will occur.[5]

$$t = \frac{(\bar{x}_1 - \bar{x}_2)\sqrt{(n_1 + n_2 - 2)}}{\sqrt{(S_1 + S_2)}} \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

In a table the value P corresponding to different values of t can be found. In this formula

$\bar{x}_1$ = mean of one set (n_1) of observations (*i.e.*, non-flowering plants),

$\bar{x}_2$ = mean of another set (n_2) of observations (*i.e.*, flowering plants),

S_1 = sum total of the square of the deviations from $\bar{x}_1$,

S_2 = sum total of the square of the deviations from $\bar{x}_2$.

In table XI the values (1–P) are given for the differences observed between the flowering and non-flowering plants.

TABLE XI

PROBABILITY (1–P) THAT A DIFFERENCE EQUAL OR LARGER THAN THE DIFFERENCE OBSERVED BETWEEN FLOWERING AND NON-FLOWERING PLANTS WILL OCCUR

PLANTS COMPARED	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES	STEMS ALONE
43 non-flowering and 43 flowering ..	0.03	0.03	0.07	0.02	0.075	0.045
43 non-flowering and 43 II + 09	<0.0003	0.077	0.0084	0.0001	<0.0002	0.0002

[5] This method was kindly suggested to me by Dr. H. HOTELLING, of the Food Research Institute.

If the value (1–P) = 0.05 the difference is suggestive
= 0.02 the difference is significant
= 0.01 the difference is very significant

Table XI shows that in crock 43 a significant difference exists between the 1st, 2d, and 4th leaves, and probably the stems of flowering and non-flowering plants, and that between the five non-flowering plants of 43 and the six plants in 09 + the flowering plant of 43 (43 II) a very significant difference exists except for the 2d leaves. That the difference in the 3rd leaves, 5th leaves and stems are not significant is due to plant 43 I, which shows rather low moisture values, though not so low as the flowering plant 43 II. This plant had not yet produced stamens, but the ratio stem length/leaf area was 1.07, which is equal to the mean of the plants which had staminated (1.06 ± 0.015) while the four other non-flowering plants in this crock showed a ratio of 0.88 ± 0.02. This proves that this plant was near to the flowering stage. If this one plant be omitted, then the values for (1–P) become much more significant. It is difficult to say whether in this plant the low value of the moisture was due to beginning of water loss or not. Moreover, our data do not show at exactly what moment the loss of water from the leaves, stems and ears occurs, whether at stamination, at fertilization of the ovaries, or earlier.

Fig. 4 and fig. 5 show graphically the dry weight and moisture per cm.^{2} of the different leaves. Fig. 5 brings out the fact that the dry weight increases as we ascend from the 2d to the 5th leaves; the smoothed values for the 1st leaf are somewhat higher than the values for the 2d leaf. Furthermore, it can be seen that after flowering the increase in dry weight in the 4th and 5th leaves is much larger than in the 1st, 2d, and 3rd leaves.

Fig. 4 shows clearly that the moisture decreases from the 1st leaf to the 5th leaf. The 1st and 2d leaves show constant values 10 days before flowering; in the 3rd, 4th and 5th leaves the values increase continuously until flowering. The slope of the curve is less steep the higher the order of the leaf. In table XII is shown the number of days from the appearance of the leaves to the time of maximum moisture content.

This table shows that the number of days during which the moisture increases is the same for the first two leaves, perhaps also for the 3rd and 4th leaves. In the 1st leaves the moisture remains constant for 12 days (19th–31st day), in the 2d leaves for 10 days (21st–31st day). This plateau does not occur in the 3rd–5th leaves as the flowering period interferes.

After flowering the drop in moisture is most pronounced and continuous in the 1st leaves; in the other leaves the decrease slows down earlier the higher their order. In this way the moisture in the 4th leaves remains

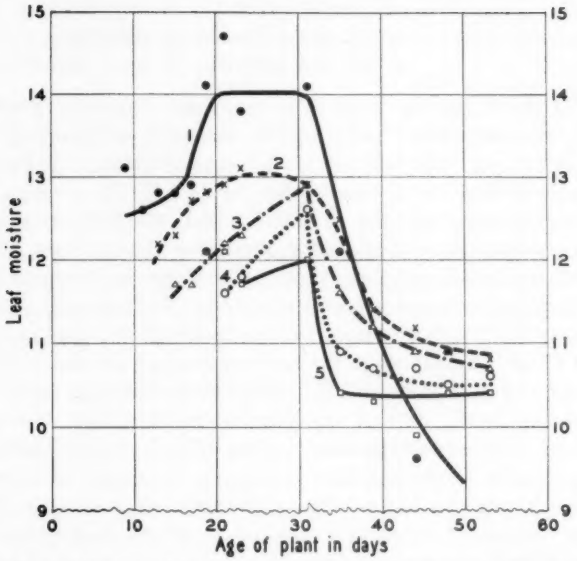

FIG. 4. Moisture of the successive leaves in mg. per cm.² leaf area. Experiment of 1926. See table VIII. The one flowering plant on the 31st day is omitted.

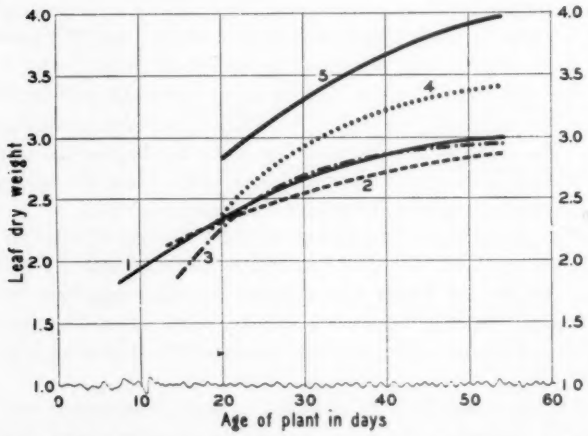

FIG. 5. Dry weight of the successive leaves in mg. per cm.² leaf area. Experiment of 1926. See table IX. The one flowering plant on the 31st day is omitted.

TABLE XII

NUMBER OF DAYS DURING WHICH THE MOISTURE IN THE SUCCESSIVE LEAVES INCREASES. EXPERIMENT 1926

LEAF NO.	DAY OF APPEARANCE	DAY ON WHICH THE HIGHEST VALUE WAS REACHED	NUMBER OF DAYS DURING WHICH MOISTURE INCREASES
1st	5th	19th	14
2d	7–8th	21st	13–14
3rd	12th	23–31st	11–19
4th	16th	23–31st	7–15
5th	19th	23–31st	4–12

about constant after the 39th day, in the 5th leaves after the 35th day. Apparently the lower leaves are more affected by the flowering than the higher leaves. It is possible that the fact that after flowering the lower leaves gain less in dry weight than the upper ones, has to be explained in this way. The external evidence of a decrease of water content is shown by the gradual yellowing and dying of the leaves. While on the 31st day 16 per cent. of the first leaves and 16 per cent. of the 2d leaves showed yellow tips, on the 35th day 66 per cent. of the 1st leaves, and 33 per cent. of the 2d leaves showed such tip. The 3rd, 4th, and 5th leaves did not exhibit yellow tips on the 35th day, though their moisture had decreased; the first yellow tips were visible on the 39th day. Therefore the yellowing of the tips cannot account for the decrease in moisture,[6] but the continuous deficit in moisture in the leaves is the principal cause of their yellowing and dying.

Determinations after the 53rd day were not made, as the lower leaves began to die rapidly and the yellowing of the upper leaves increased.

Discussion

The tables given in this paper show plainly that in the different cases a change in percentage of dry weight must be explained in different ways. The fact that the higher leaves show a higher dry weight per cent. is due to their higher dry weight and lower moisture per unit leaf area. The dry weight per cent. of every leaf increases throughout the life cycle. Before the flowering stage this is due to the fact that both dry weight and moisture increase, but the former increases more rapidly than the latter; after the flowering stage the moisture decreases while the dry weight continues to increase. Probably exactly the same holds for the "stems alone." In

[6] Even if these tips had contained no water at all, this could not account for the absolute quantity of water loss that was observed.

experiment 1925 the dry weight per cent of the successive internodes was determined on the 48th day, *i.e.*, about 9 days after flowering.

TABLE XIII

PERCENTAGE DRY WEIGHT OF SUCCESSIVE INTERNODES (48TH DAY, EXPERIMENT 1925)

1st internode + 1st sheath + 2d sheath + coleoptile	29.0 per cent.
1st node + 2d internode + 3rd sheath	22.0 per cent.
2d node + 3rd internode + 4th sheath	23.3 per cent.
3rd node + 4th internode + 5th sheath	29.5 per cent.

Though probably the first internode always shows a somewhat higher dry weight per cent. than the 2d internode (as is the case in the 1st and 2d leaves, fig. 5), the high value of the former in table XIII is certainly due to the fact that this internode loses more water after flowering than do the higher internodes (as is also the case in the leaves, fig. 5). This corresponds with the fact that the first leaves with sheaths, and the 1st internodes, had already begun to yellow at this time.

This analysis of dry weight per cent. data shows that a change in this value can be brought about very differently in different cases. It is strictly necessary to express the total absolute moisture and dry weight or to calculate the values per unit length or area. This analysis shows furthermore that it is not permissible to compare the dry weight per cent. of leaves in different plants without taking into consideration the order of the leaves.

In experiment 1926 the temperature was about 4° C. higher than in 1925; therefore transpiration must have been much higher in 1926. The dry weight per cent. in 1926 was always higher than the same value in 1925. Thus this value 1–2 days before flowering was 16.1 per cent. in experiment 1925 (38th day); 19.5 per cent. in experiment 1926 (five non-flowering plants 31st day). After flowering, these differences between the values in both experiments persisted. In 1926 the leaves and ears emerged earlier; the leaf area and stem length were less than in 1925. The rate of growth of the stems, however, seems to have been nearly the same in both experiments, as in 1925 a final length of 79 cm. was reached in 39 days, in 1926 a length of 60 cm. in 30 days. Therefore the differences between the two experiments must be ascribed in part to a difference in growing period between sowing and flowering. But parallel with a shorter growing period there goes a more rapid increase of the percentage of dry weight. We think that this higher percentage of dry weight (or lower percentage of moisture) is the cause of the shortening of the period between sowing and flowering. The dry weight per cent. is certainly an important physiological index. In our experiment it was very uniform in different plants of the

same age. In 1925 this value for the entire top was 14.0 ± 0.10 per cent. (average of nine plants) on the 30th day.

The dry weight of the leaves shows a continuous increase throughout the life cycle. Though the leaves may be full grown insofar as a constant leaf area is reached, they still increase in weight. DIXON (**4**) found that older leaves showed a higher osmotic pressure than younger leaves. URSPRUNG and BLUM (**16**) found the osmotic value (saltpeter value) in the younger leaves of *Urtica* smaller than in older ones; the value in the stem of *Urtica* and *Helleborus* decreases from the base towards the top. WILLSTÄTTER (**15**) showed that the chlorophyll content of leaves may increase for several weeks. The explanation of the different dry weight and moisture of the successive leaves must wait until further anatomical studies have been made. The marked sequence of the values for the dry weight and moisture of every leaf and their low probable errors proves that in different plants the leaves of the same order are very uniform in structure and physiological behavior. In other words, the sampling error has been practically eliminated.

While the first parts of the dry weight curves of the successive leaves are curves with increasing slope, the last parts of these curves, as represented in figs. 4 and 5, exhibit a decreasing slope (S-shaped curve).

The increase in dry weight throughout the life cycle is also found in the stems. Though the higher internodes are thinner, the average dry weight per cm. stem length increases continuously. This increase lasts for a certain time after flowering, as is also the case in the leaves.

The increase in moisture and dry weight in the leaves and stems which have completed their increase in area or length is probably due to a further increase in volume of the parenchyma cells.[7]

The moisture in the stems and in the leaves increases continuously until the flowering stage interferes. After flowering, with the increase of dry weight in these organs, there is no longer increase in moisture, but the opposite, *viz.*, a sudden loss of water. That after flowering the lower leaves gain less in dry weight than the upper ones may be explained by the fact that the lower leaves suffer more from water loss than the upper (table IX and fig. 5). Indeed, several investigators [literature cited in (**2**)] have shown that photosynthesis depends largely upon the water content of the leaves. On the other hand, it is possible that the lower leaves were physiologically full grown, while the upper leaves, developing later, are still capable of increasing in weight. Owing to a more pronounced and continuous deficit in water content, the lower leaves are the first to die.

The same probably holds for the stems in which the first internode is the first to yellow.

[7] This has been pointed out by BERTHOLD (Unt. z. Phys. der Pflanzl. Organization **2**: I. 1904.

The water loss in the ears lasts only a few days as the kernels begin to swell, resulting in a marked increase in dry weight and moisture.

The increase in dry weight in the different organs will be discussed in a later paper. We may, however, anticipate so far as to state that the loss of water in the leaves and other assimilatory organs must affect photosynthesis, thus depressing the total increase in dry weight. In the field this should be the more pronounced the drier the soil and air. Moreover, in the yellowing leaves the rate of photosynthesis must fall off. This results in a decrease in the slope of the growth curve (last part of the total S-shaped curve), which is the more accentuated as the leaves gradually die from the base towards the top (**1**).

The cause of this sudden loss of water at the time of flowering or fertilization must now be considered. Experiments with sand and with water cultures indicated that the increase in transpiration, corresponding to the growth of the last internode, ceases 1–2 days before the plants produce stamens. The loss of water probably occurs 1–2 days after flowering. It is not probable, therefore, that the loss of water is due to the fact that the ears while growing came nearer to the lamps resulting in an increase in transpiration.[8] Moreover, in crock 43 the flowering plant (61.1 cm. long) was not the tallest one, two non-flowering plants were respectively 64.3 and 60.4 cm. long while their moisture was practically equal to or higher than the mean of the non-flowering plants. Moreover, several plants after flowering were shorter than 59.6 cm. (the average of the five non-flowering plants in crock 43), but showed nevertheless a pronounced loss of moisture. Furthermore, the loss of water at this time is certainly a general phenomenon in annuals grown in the field as data in the literature show (**2**). Therefore, the flowering stage must be an important moment in the life cycle of an annual or biennial and we might expect cloudy or rainy weather to be favorable at this time (**1**). On the other hand, rain may be harmful at this stage through washing nitrates, etc., out of the soil, thus complicating the answering of the question whether or not the flowering stage is a critical period in the life cycle. At any rate, the fact that the drying out of an annual begins as soon as the young sporophytes begin to develop must be considered.

The growth in length of the internodes ceases on the same day the stamens appear, *i.e.*, when pollination takes place. A similar phenomenon was studied by MURNEEK (**9**) in tomatoes. He found that vegetative growth was inhibited by developing fruits. This inhibition was more pronounced the greater the number of the fruits that were developing. There

[8] The lamps were renewed in both experiments at such a time as not to interfere with the flowering period.

is, however, a difference between the case of wheat and of the tomato. The fruits of tomatoes are lateral, in wheat apical; the inhibition of vegetative growth in tomatoes occurs while the fruits are developing, in wheat at flowering. MURNEEK ascribes this phenomenon in tomatoes to the fact that the fruits monopolize the nitrogen. As was pointed out in a previous paper (**2**), it is preferable to say that the cells in the fruits are in such a state that they can take in and utilize nitrogen whereas the cells in the vegetative parts cannot. That these parts are still able to grow is proven by the fact that this inhibition depends to a large extent upon the nitrogen given to the plant. Growth is resumed also if the fruits are removed. On the other hand, vegetative growth in flower shafts of Composites and Crucifers (**13**) and in the coleoptiles of *Avena* (**12, 5**) and of other cereals is only possible if a growing point or a mass of meristematic tissue is left. If the flower heads (*Compositae*), inflorescence (*Cardamine*) or the meristematic tip (*Avena*) are removed, the growth in length is inhibited.[9] The same is true in wheat. In preliminary experiments in the field in some plants the ear was cut off some time before flowering. The loss of water from the cut was lessened either by putting a paper cap around it or cutting the ear while still in the boot. The growth during the first two days after cutting of the controls was 54 mm. (average of four plants), that of the plants without ear 22.5 mm. (average of five plants), the next two days it was respectively 60 and 7 mm.

If in the experiments just cited the top of a cut flowerstalk or coleoptile was replaced on the stump, growth increased again. There is an abundance of evidence in recent literature (**10, 14,** further literature cited there) that from the growing point or active meristematic tissues a substance diffuses downward, which is usually called a "growth-promoting substance" or "growth hormone," *i.e.*, a substance which makes growth possible. These terms do not imply anything as to its chemical character. If the meristematic tissue is removed, no such substance flows down, thus preventing the growth of cells which otherwise would be capable of increase in size. This is the case in plants from which the apical meristematic tissue (tip of coleoptile, young flowers or ears) has been removed. As soon as fertilization takes place, the embryo and the seed begin to have an independent life and act as independent meristematic tissues. If we assume that the growth-hormones formed inside the developing ovules and fruits do not leach out, we can explain why the growth in length of the flower shaft stops[10] after fertilization; as for the plant, the apical growing point has virtually ceased to exist. The young embryo or embryos form, however, new growth-hormones, which are confined within the fruit, making its growth possible.

[9] The formation of a new physiological tip may complicate this phenomenon (**12, 5**).

[10] In cases of a double growth period, we have to assume a partial leaching out of these substances.

Another hypothesis which has been advanced in order to explain the correlation between growing points and the growth of cells below it (or, on the other hand, the disturbing of this correlation by removing the growing point), is the assumption of a suction force ("Saugkraft") developed by the growing point. In this theory the growing point is supposed to be a center of attraction for foodstuffs (**13**). The monopolization of nitrogen and carbohydrates by the fruits of tomatoes and cotton (**8**) is also an example of the attraction or diversion of foodstuffs by the developing fruits from the growing vegetative cells.

These two theories—*viz.*, the theory of the growth-hormones secreted by the growing point and the theory that the foodstuffs are monopolized by the young fruits—are, however, not incompatible, but can be brought together in a more general theory of growth. This is possible if we assume that the growth-hormones have a lyophilic effect upon, or increase the swelling-capacity of the colloids in the protoplasm of the young growing cells, and that they prevent the dehydration of the colloids in the protoplasm of the older cells. As soon as growth-hormones no longer diffuse from the apical growing point down into the vegetative parts below it, as may result from decapitation or fertilization, vegetative growth must stop. But then the imbibition-capacity of the colloids in the older cells must also decrease and water must be lost at this period and later. In this way the cessation of growth in length, the loss of water from the various organs, and the gradual dying of the leaves after flowering can be explained. How the action of the hormones upon the imbibition-capacity of the plasma colloids of a young cell may result in its growth and how this theory places greater importance upon the protoplasm and its hydration than upon the osmotic pressure of the cell sap, I hope soon to show.

Summary

A pure strain of Hard Federation wheat was grown under constant conditions. Ripe (air dry) seeds were obtained in 72 days. Every plant, when full grown, had five leaves and one single stem and ear. The moisture of the different organs throughout the life cycle was determined by harvesting six plants every other day, later on every 4th or 5th day. Sampling errors could be eliminated to a large extent by calculating the moisture and dry weight per unit stem length or per unit leaf area and also by calculating the probable errors.

From the lowest to the uppermost leaf, the dry weight per unit leaf area increases, while the moisture decreases. Throughout the life cycle the dry weight of the leaves increases; the moisture increases until the time of flowering. A sudden drop of moisture occurs in all the organs of the plant 1–2 days after the stamens have appeared. This drop is most pronounced

in the lower leaves, sheaths and internodes. The higher leaves lose less water and are thus able to persist longer than the lower ones. The first sign of this water loss is the yellowing of the tips of the leaves. Therefore the drying of the plant, which leads to the final death of the annual, begins as early as 1–2 days after pollination, *i.e.*, about the time of fertilization.

As an explanation of this loss of water from the different organs of the plant after flowering the theory is advanced that the imbibition-capacity of the plasma colloids, which through the action of the growth-hormones is kept up as long as a vegetative growing point is present, decreases as soon as, through the act of fertilization, this growing point is virtually lost to the vegetative plant.

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[1] After this manuscript had been sent to press a publication by F. W. WENT appeared, entitled "Wuchsstoff und Wachstum," Dissertation, Utrecht, 1927, which places the existence of growth hormones beyond doubt.

NITROGEN METABOLISM IN THE SOY BEAN*

J. E. WEBSTER

Introduction

In attempting to throw light on the protein metabolism of green plants, the variations in the quantities of the simpler water soluble nitrogen compounds of the soy bean (*Glycine max* var. Peking) (**8**) at various stages in its life cycle were determined and the effects of different conditions of nitrogen supply were studied.

The soy bean was selected for this work, since it is of medium height, is high in nitrogen and matures relatively early.

EMMERLING (**3**) reviewed the earlier literature of this subject very carefully and gives an interesting discussion of his own work with *Vicia faba*. He analyzed plants on nine different dates, making separate analyses of various parts such as the roots, stems, leaves, pods and seeds. He found that the seeds secured their nitrogen at the expense of the entire plant and that the leaves and hulls seemed to act as reserve storage organs for amides. A discussion of the literature on this subject up to a somewhat later date is given by SCHULZE (**10**). SURE and TOTTINGHAM (**13**) concluded from a rather complete fractionation of the nitrogen of etiolated plants that α-amino-acids served for the production of amides in the nitrogen metabolism of etiolated plants.

CHIBNALL (**2**) has made quite extensive studies on nitrogen metabolism, especially of the leaf of the runner bean. He found that the leaf proteins changed but little after formation and that the fluctuations which occurred in the nitrogen compounds were mainly of the soluble forms. Some question has been raised by NIGHTINGALE (**5**) after his study of protein metabolism in various plants grown under different conditions particularly of light, as to the influence of the soluble forms of nitrogen on plant growth and he suggests that perhaps the ratio of insoluble nitrogen to carbohydrates is one of the most important in plants.

STROWD (**12**) analyzed the leaves, stems, roots and nodules of soy beans grown under field conditions. He concluded that there was an increase in the nitrate content of the roots during arrested photosynthesis and that nitrates increased in the sap, but not proportionally, when the nitrate supply was increased.

* Investigations pursued at the Ohio State University.

JODIDI (**4**) working with etiolated seedlings of corn followed the decomposition of the proteins in the seedlings by analyzing for various water soluble forms of nitrogen. He finds that there is a steady increase in amide and amino nitrogen and a decrease of peptide nitrogen. However, the increase in amide nitrogen is not proportionally greater than that of the amino nitrogen.

Methods of analysis

Sampling.—The procedure of sampling required about one and one half hours. The leaves with their petioles were removed, weighed and ground. After any cotyledons were removed the stems were cut off at the surface of the soil or sand and weighed and ground. Finally the roots were removed, washed free of adhering soil or sand particles, dried by careful pressing between filter paper, weighed, and ground.

Grinding and extracting.—As soon as weighed, the parts sampled were ground through a Nixtamal mill. Leaves were ground once; stems and roots two or more times depending on the coarseness. This reduced the leaves to a pulp and the stems and roots to a pulp containing shreds of fibrous material.

Before any water was added two separate portions were removed from each sample for moisture and total nitrogen determinations.

The remainder was then stirred with cold distilled water and the watery mass transferred to a filter cloth of closely woven muslin and allowed to drain. After this it was squeezed dry by hand, washed once, placed in a beaker, covered with boiling distilled water and set on a boiling steam bath for two hours. It was then cooled to room temperature and the water extract decanted. The residue on the cloth was washed thoroughly with cold distilled water and squeezed dry. This operation was repeated several times so that the material retained by the cloth and fibrous material was reduced to a minimum.

This method was found to remove practically all of the water soluble forms of nitrogen. Similar results have been secured by TOTTINGHAM, SCHULTZ, and LEPKOVSKY (**15**) who, after a comparison of various types of extraction, concluded that the water extraction method was as satisfactory as any.

Clarifying.—Before the various determinations could be made on this extract it was neecssary to remove the colloidal material. To accomplish this, 50 cc. portions of the well-shaken extract were heated to 65°–70° C. for the leaves and stems, and to 70° or even 75° C. for the roots if the lower temperature did not flocculate the colloidal material. These flocculated samples were then centrifuged until the supernatant liquid was clear, after which they were decanted and the flocculum broken up with 10 cc. portions

of water and centrifuged twice more. Two separate samples were coagulated and then the whole, including washings, was made up to 200 cc.

These extracts, after samples for ammonia and amide determinations had been removed, were preserved with toluene until the amino nitrogen determinations were made.

Moisture.—As previously stated, two samples for moisture determinations were removed at the time of grinding, and placed in paired watch glasses. These, after being weighed, were placed in a vacuum oven which was held constant at 70° C. and through which a continuous small stream of air was drawn. The samples were allowed to remain in the oven for five days after which they were removed, weighed, covered with water and allowed to soak for a day. They were then transferred to Kjeldahl flasks to be used for total nitrogen determination.

Total nitrogen.—The moisture samples after being transferred to Kjeldahl flasks were placed on a steam bath and evaporated to dryness under reduced pressure (**9**). The samples were then analyzed according to the official Gunning method, modified to include the nitrogen of nitrates (**6**).

Soluble nitrogen.—Duplicate determinations were run on 50 cc. portions of the original extract after it had been clarified (see clarifying). These determinations were made in the same manner as the total nitrogen samples above.

Insoluble nitrogen.—This percentage is secured by difference (total–soluble).

Ammonia nitrogen.—For this determination a modified Folin's method was used. Ten cc. samples of the clarified solution, 15 cc. of 52 per cent. K_2CO_3 and 5–8 drops of capryl alcohol were added to the tubes on one side of the VAN SLYKE-CULLEN urea apparatus. To the tubes on the other side were added 10 cc. of N/50 acid, 15 cc. of water, 6 to 8 drops of capryl alcohol and 3 or 4 drops of methyl red indicator. The apparatus was then connected and aerated at such a rate as would draw over 120 liters of water in an hour. The excess acid was then titrated in the tubes, using N/50 alkali.

The results were calculated after subtracting the titration figures from those secured by running blanks. To secure the figures shown in the tables four determinations were made on each sample and the results averaged to get the final figures.

Amide nitrogen.—For these determinations an acid hydrolysis method suggested by PHILLIPS (**7**) was used. Ten cc. portions of the sample were transferred to tubes of the VAN SLYKE-CULLEN apparatus, several small pieces of pumice and 0.6 cc. of concentrated H_2SO_4 added, and the sample boiled gently under a reflux for two and one-half hours. The samples

were then cooled, nearly neutralized with 2.2 cc. of 20 per cent. NaOH, then cooled again, and capryl alcohol and 20 cc. of 52 per cent. K_2CO_3 added. The system was aerated as in the ammonia determination. Four determinations were made on each sample and the results averaged. The difference between the ammonia titration and the amide titration gives the acid equivalent to the ammonia secured by hydrolysis.

Nitrate nitrogen.—The method used was a modified DEVARDA'S alloy method. The method suggested by STROWD (**11**) has been found to be unreliable under certain conditions (**1**) so that a modification by PHILLIPS was used (**7**).

The residue from the amide determination was washed into an 800 cc. Kjeldahl flask using 100–135 cc. of water. This was then boiled nearly to dryness, allowed to cool, one and one-half grams of DEVARDA'S alloy added and the whole diluted with 300 cc. of distilled water. The samples were then distilled into N/50 acid containing methyl red as indicator. The excess acid was titrated with N/50 alkali after the samples had been boiled to expel CO_2 and cooled. Blanks were run using 20 cc. of 52 per cent K_2CO_3 instead of the nitrate samples.

Nitrite nitrogen.—The amount present in any case is very small; however, for a rough qualitative test the GRIESS-ILOSVAY method (**14**) was used.

Amino nitrogen.—This determination was made on the cleared solution (see clarifying) using the VAN SLYKE apparatus. No correction was made for the ammonia present.

Other nitrogen.—This percentage was secured by adding together the amino, nitrate, and ammonia results and subtracting this total from the percentage of soluble nitrogen.

Experimental

Due to the large amount of analytical material secured, no attempt will be made to present it all in table form, but only such data as will illustrate the work and show a few of the major points. The summaries for such series as are not represented by tables, were secured by a careful study of the analytical results and of graphs representing the same material. All statements in this paper referring to amounts of the various forms of nitrogen should be understood as refering to percentages of nitrogen in terms of fresh weight of the plant organ analyzed. Also the percentages represent the analyses of at least thirty plants, and in the case of smaller plants, one thousand or more.

SERIES A

HIGH NITROGEN PLANTS GROWN IN SOIL.—These plants were all grown in the greenhouse during the first four months of 1925. This series was con-

tinued for sixteen weeks by the end of which time the plants were mature and the seeds had begun to drop from the pods. The seeds and pods were discarded in all these series. The soil was a rich humus type contained in twelve-inch unglazed pots. The soil had been sterilized before using and at no time were there any nodules observed on the roots. Due to the large number of plants in the pots intended for the first six weeks, a dilute nitrate solution was added to each (one tablespoon of KNO_3 to eight liters of water).

The variations in the percentages of total, insoluble and soluble nitrogen in the leaves, stems and roots are quite comparable with one another with the exception of the soluble percentage in the leaves. The curves for total and soluble nitrogen forms, show two low points; one, two to three weeks after germination when the stored reserve nitrogen seems to be greatly depleted, and before the plant has itself begun to elaborate many nitrogen compounds; the other, at the stage when the seeds are nearly full grown.

The percentages of nearly all forms of nitrogen fluctuate greatly at the sixteenth week when notable degeneration has begun.

The nitrates as a rule are much higher in the stems and roots than in the leaves. The relation between the various parts is in general similar, with the roots showing the greatest discrepancies. The various parts of the plant are lowest in nitrates at about the time of seed maturity (tenth to twelfth week).

Curves for the amino nitrogen run nearly parallel in the case of the leaves and stems but those of the roots diverge widely from the other two. Amino nitrogen is lowest at the inception of visible flowering, and highest at the period when the plants are nearly mature.

There is no striking comparison between the curves for amino and amide nitrogen although it is true that any great variation in the amino is usually reflected in the amide percentage. There is no evidence to show that either the amide or the amino forms of nitrogen are drawn upon for the production of seeds, resulting in a noticeable depletion of that form of nitrogen in any organ, as has been suggested by some writers. The ammonia figures are lowest around the eighth to tenth week and highest during the later weeks when degeneration is greatest. Nitrites are present in the leaves during the earlier stages but their presence in other parts is doubtful.

"Other nitrogen" figures are quite variable and at times show a negative value, possibly due to an incomplete recovery of nitrates in the soluble fraction. There is, however, a remarkable increase in this form in the stems after the tenth week.

Series B and C

High nitrogen series B and low nitrogen series C.—These plants were grown in the greenhouse during the months of May, June and July. They were grown in clean quartz sand contained in two-gallon glazed jars which were set in glazed saucers that were kept about one-half full of water. Only distilled water was used in watering plants grown in quartz sand.

The nutrient solution used was made up of the following pure chemicals dissolved in distilled water:

$MgSO_4 \cdot 7H_2O$	2 per cent.
KNO_3	2 per cent.
$Ca(NO_3)_2 \cdot 4HO_2O$	10 per cent.
K_2HPO_4	2 per cent.

For each pot of the high nitrogen plants, 25 cc. of each of the above solutions were mixed with 5 cc. of a 2 per cent. solution of iron citrate and the whole made up to 250 cc. and added weekly.

The solution used for the low nitrogen pots was identical with the high nitrogen one, with the exception that only 5 cc. of the 10 per cent. $Ca(NO_3)_2 \cdot 4H_2O$ was used.

Great difficulty was experienced in keeping green algae out of the saucers and usually after two or three weeks in the greenhouse the saucers were covered with them. The results of the analysis of the high nitrogen series B are shown in tables I, II and III, and of the low nitrogen series C in tables IV, V, and VI.

Comparison of series B and C.—While a difference in the level of nitrogen supply does markedly influence the physiological appearance of the plants, in this case at least, it does not seem to have greatly affected the growth habits.

The main chemical differences are shown in the considerably lower total nitrogen content of the low nitrogen plants and the presence of nitrites in the stems and roots at only a few periods. One other striking feature of the low nitrogen plants is their very much lower ratio of amide to amino nitrogen as compared to the same ratio in the high nitrogen plants. This statement is true for the leaves, stems, and roots. The percentage of moisture is also slightly higher in the low nitrogen plants.

Series D

Low nitrogen plants grown with a nutrient solution lacking nitrates.—These plants were grown in a manner similar to that used in the

TABLE I

PERCENTAGE OF FRESH WEIGHT, LEAVES

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.9781	0.5127	0.4654	0.0815	0.1344	0.0464	0.0185	÷÷÷:	0.2310	81.59
2	0.7820	0.5510	0.2310	0.0901	0.0759	0.0147	0.0091	÷÷	0.0559	87.44
3	0.7080	0.5301	0.1779	0.0773	0.0610	0.0101	0.0058	÷÷	0.0338	87.88
4	0.7342	0.5833	0.1509	0.0299	0.0598	0.0119	0.0054	÷÷	0.0558	85.07
5	1.010	0.8527	0.1573	0.0271	0.0602	0.0134	0.0028	÷÷	0.0672	84.09
6*	0.8950	0.7045	0.1905	0.0526	0.0745	0.0140	0.0027	÷÷:	0.0607	83.16
8	0.6771	0.5554	0.1217	0.0189	0.0498	0.0093	0.0054	÷	0.0476	82.02
10	1.0007	0.7896	0.2111	0.0261	0.1039	0.0123	0.0138	none	0.0673	75.83

* First blooms were beginning to appear.

TABLE II

PERCENTAGE OF FRESH WEIGHT, STEMS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.5758	0.1583	0.4175	0.0577	0.1912	0.1399	0.0116	faint	0.0630	92.75
2	0.4142	0.0921	0.3221	0.1365	0.1696	0.0579	0.0064	faint	0.0096	91.55
3	0.2595	0.0491	0.2104	0.1374	0.0454	0.0243	0.0030	?	0.0246	90.72
4	0.1880	0.0880	0.1000	0.0416	0.0325	0.0110	0.0072	faint	0.0187	89.11
5	0.2367	0.1441	0.0946	0.0408	0.0468	0.0118	0.0025	÷÷	0.0045	88.30
6	0.2341	0.0982	0.1385	0.0764	0.0387	0.0171	trace	÷÷	0.0234	85.62
8	0.1765	0.0788	0.0997	0.0212	0.0530	0.0092	0.0043	÷	0.0215	81.99
10	0.3013	0.1599	0.1414	0.0270	0.0680	0.0327	0.0100	none	0.0364	75.03

TABLE III

PERCENTAGE OF FRESH WEIGHT, ROOTS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.2478	0.0772	0.1706	0.0215	0.0842	0.0285	0.0157	÷	0.0492	95.66
2	0.2059	0.0491	0.1568	0.0868	0.0425	0.0069	0.0131	÷	0.0144	95.29
3	0.1795	0.0759	0.1036	0.0631	0.0173	0.0079	0.0094	faint	0.0148	94.54
4	0.1614	0.0821	0.0793	0.0340	0.0212	0.0045	0.0019	faint	0.0222	90.76
5	0.1834	0.1201	0.0633	0.0307	0.0294	0.0027	0.0032	÷	0.0000	91.08
6	0.2069	0.1252	0.0817	0.0456	0.0241	0.0066	0.0027	÷÷÷	0.0093	90.46
8	0.1526	0.1137	0.0389	0.0203	0.0177	0.0036	0.0015	÷	0.0006	89.03
10	0.1758	0.1044	0.0714	0.0270	0.0247	0.0071	0.0099	faint	0.0098	88.86

TABLE IV

PERCENTAGE OF FRESH WEIGHT, LEAVES

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	1.2006	0.8000	0.4006	0.0364	0.1260	0.0436	0.0164	÷÷	0.2218	82.82
2	0.7013	0.5189	0.1824	0.0478	0.0559	0.0142	0.0099	÷÷	0.0688	87.42
3	0.6112	0.4632	0.1480	0.0506	0.0337	0.0073	0.0063	÷÷	0.0574	88.18
4	0.5612	0.4501	0.1101	0.0242	0.0646	0.0099	0.0056	÷	0.0157	85.66
5	0.6353	0.4977	0.1376	0.0067	0.0704	0.0094	0.0022	÷	0.0583	83.76
6	0.4508	0.3817	0.0691	0.0152	0.0471	0.0063	0.0057	none	0.0011	79.45
8*	0.5146	0.4382	0.0764	0.0104	0.0319	0.0068	0.0026	none	0.0315	81.31
10	0.4703	0.3478	0.1226	0.0176	0.0614	0.0179	0.0081	none	0.0355	77.61

* First blossoms were appearing.

TABLE V

PERCENTAGE OF FRESH WEIGHT, STEMS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.5680	0.1713	0.3967	0.0217	0.1913	0.1359	0.0067	÷	0.1770	92.88
2	.....	.....	0.2379	0.0916	0.0612	0.0533	0.0071	÷÷	0.0780	91.63
3	0.2406	0.0448	0.1958	0.1119	0.0379	0.0200	0.0100	÷	0.0360	90.40
4	0.1577	0.0646	0.0931	0.0386	0.0473	0.0073	0.0044	÷	0.0028	82.29
5	0.1660	0.0768	0.0892	0.0164	0.0574	0.0103	0.0018	none	0.0136	84.58
6	0.1507	0.1100	0.0407	0.0079	0.0458	0.0055	0.0012	none	− 0.0142	76.80
8	0.1377	0.0734	0.0643	0.0192	0.0398	0.0073	0.0079	none	+ 0.0450	88.08
10	0.1748	0.1015	0.0733	0.0345	0.0350	0.0076	0.0106	none	− 0.0068	76.61

TABLE VI

PERCENTAGE OF FRESH WEIGHT, ROOTS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.2373	0.0920	0.1453	0.0052	0.0707	0.0332	0.0128	÷÷÷÷	0.0566	95.67
2	0.1188	0.0500	0.0688	0.0279	0.0274	0.0068	0.0056	÷	0.0079	95.54
3	0.1877	0.1107	0.0770	0.0351	0.0172	0.0035	0.0063	faint	0.0184	95.76
4	0.1388	0.0854	0.0534	0.0224	0.0230	0.0027	0.0036	faint	0.0044	92.45
5	0.1442	0.0949	0.0493	0.0105	0.0298	0.0066	trace	faint	0.0090	92.08
6	0.1457	0.0896	0.0561	0.0060	0.0145	0.0012	0.0023	faint	0.0333	90.60
8	0.1330	0.0688	0.0642	0.0169	0.0189	0.0028	0.0031	none	0.0252	80.61
10	0.1315	0.0799	0.0516	0.0196	0.0231	0.0041	0.0053	none	0.0036	89.98

two preceding series and differed only in the use of the following minus-nitrate nutrient solution:

$MgSO_4.7\,H_2O$	2 per cent.
KCl	2 per cent.
$CaCl_2.2\,H_2O$	4 per cent.
K_2HPO_4	2 per cent.

Samples were taken when the plants were two, four and six weeks old. The analyses of this series show but one significant fact; namely, that there is a constant decrease of nitrogen in all parts of the plant and that this decrease is shared alike by all of the forms for which analyses were made.

Series E

Low nitrogen plants grown in quartz sand to which no nutrient solutions were added.—The plants of this series were grown during July and August in quartz sand prepared in the same manner as that in B and C. These, however, were not given any nutrient solution and consequently were dependent upon the material stored in their cotyledons for growth.

The total and soluble nitrogen curves for the leaves, stems and roots show very much the same fluctuations, there being, after the second week, a small continual fall in each. The insoluble nitrogen, however, is quite variable. Points of particular interest are the increase in the amino, amide, and ammonia forms of nitrogen at the second week and the small change in the insoluble fraction at all times in the leaves and stems.

The various soluble forms with the exception of the amino nitrogen at the sixth week show somewhat of a drop after the second week.

The trace of nitrites present in the various parts at the end of the first week is interesting, although no explanation of their presence can be given.

It is also worth noting that compared with series D, in which the percentages of all forms fall off rapidly in the leaves and stems, there is only a slow decrease, in these organs, in the percentage of total nitrogen; and this decrease occurs almost entirely in the soluble fraction. In the roots, however, the decrease is shown equally in both fractions.

Summary

Series A presents data for plants grown in humus soil, in pots in the greenhouse. These plants were sampled weekly. Series B and C were grown in sand cultures in the greenhouse and differ only in the amounts of nitrates applied, series B having received the larger amount. Series D and E were grown similarly to B and C and differ only in the type of nutrient solution supplied; D contained no nitrates and E received only distilled water.

In series A, B and C there is a weekly variation of percentages of total nitrogen in no particular direction. This would not be taken into account by data representing only one or two samplings during the life cycle of the plant and seems to be a point that has been overlooked by many in interpreting chemical analyses of plants.

The fact that the supply of nitrogen may be varied over such wide limits as shown in series B and C and yet not seriously disarrange the growth cycle is of interest in discussing the carbohydrate-nitrogen ratio. Data concerning the carbohydrate fluctuations would, however, be necessary before this could be discussed fully.

From a consideration of the first series, A, and some data unpublished it seems that the addition of nitrates results in a temporary increase in nitrate storage in all organs of the plant. This is soon, however, reduced to normal without any noticeable increase in the other soluble forms.

It is worth noting that the soluble nitrogen is relatively much more abundant in the stems and roots than in the leaves of all the plants for which data are presented.

There is no evidence to show that, under these conditions, there is any tendency for any of the forms of nitrogen here considered to become grouped in a particular organ of the plant with a resultant loss to any other organ.

It must be borne in mind that in considering these data the plants were grown under greenhouse conditions and this may account for some of the discrepancies between this work and that reported for some field work by other investigators.

On the basis of the amount present, amide forms of nitrogen do not seem to be of any more importance in nitrogen metabolism of plants than are the amino-acids. There does, however, seem to be a tendency for the ratio of amide to amino nitrogen to increase when a large supply of nitrogen is available to the plant. This might be interpreted as showing that amide forms occur as soluble storage forms.

It seems possible that the formation of nitrites may represent an intermediate stage in nitrogen metabolism when nitrates are supplied to plants. On the other hand, nitrites do not seem to occur in detectable amounts when organic forms of nitrogen are used or when plants are living on their stored reserves.

Although the percentages of ammonia are very small, in each series there seems to be reason for concluding that ammonia is one of the intermediate stages in both the synthesis and destruction of proteins in plants.

The column marked "Other nitrogen" represents a nearly unknown fraction of soluble nitrogen. It is hoped, when time is available, to carry on work leading to a fraction of this portion.

Conclusions

1. In general, the variations of the various types of nitrogen compounds in the roots, leaves and stems of any particular series are quite similar.

2. There is little or no evidence to support the idea that the leaves, stems, or if present, the seeds, draw upon the nitrogen reserves of the roots.

3. Amino-acids having amide groupings, on the basis of the amount present, are of no more importance in protein metabolism in plants than other amino-acids, although there is some evidence to show that amide nitrogen may be one of the most usual soluble storage forms.

4. Additional proof has been found for the idea that ammonia is the first and last product of nitrogen metabolism in plants.

5. There is considerable daily fluctuation of the various forms of nitrogen in no uniform direction.

6. Within a wide range, when the external nitrogen supply is the only variable, only the general appearance and not the physiological cycle of the soybean is changed.

The writer wishes to express his indebtedness to Dr. T. G. PHILLIPS for suggesting the problem and the helpful criticism he has given; to Dr. R. C. BURRELL for his suggestions and criticisms; and to Dr. WILLIAM CROCKER for the aid and use of the equipment of the Boyce Thompson Institute for Plant Research where the analytical part of this problem was carried out.

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THE EFFECT OF ACCUMULATED CARBON DIOXIDE ON PLANT RESPIRATION*

J. J. Willaman and J. H. Beaumont

In a study of the biochemical basis of winter hardiness in apple trees, the writers have had occasion to measure the relative rates of respiration of tender and hardy varieties. In the first series of measurements (**4**) continuous aspiration was employed; that is, during the period of measurement CO_2–free air was continuously passed through the chamber containing the twigs.

Because of certain mechanical difficulties in this method over long periods, discontinuous aspiration was then tried; that is, the CO_2 was allowed to accumulate in the chamber and was measured at the end of the period. It became apparent that an entirely different picture of respiratory activity was obtained by this procedure. Therefore, a more detailed study of the phenomenon was undertaken, the results of which are presented in this paper.

As long ago as 1881 Müntz (**16**) reported that grain emits many times as much CO_2 when it has access to fresh air as when it is confined in a container. Mangin (**15**) in 1896 pointed out that the respiration of germinating seeds is decreased by the presence of 5 per cent. of CO_2, and that the respiratory quotient becomes greater. Czapek, in his excellent review of plant respiration (**12**), does not discuss this particular phase of it. Many studies on respiration have been conducted by both methods. The well-known Osterhout method (**19**) is of the continuous type. Bailey and Gurjar (**1, 3**) used the discontinuous method on grains, conceding that it indicated a rate that continually decreased with time. Spoehr and McGee (**23**) described quite definitely the effect of changing plants from one concentration of CO_2 to another, which in effect is really the procedure in the discontinuous method. Olney (**18**) used the latter method on bananas, while Bergman (**6**) employed the continuous on cranberry plants. The present writers used the continuous method in the work on apple twigs reported preliminarily (**4**). Thomas (**25**) has found that concentrations of CO_2 above 12 per cent. in the air surrounding apples tend to increase the production of ethyl alcohol and of acetaldehyde; in other words, to change the respiration to a zymasic type.

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In the light of the facts briefly reviewed above, it is evident that the accumulation of CO_2 in the atmosphere surrounding plant tissue has an appreciable effect on the respiration of that tissue.

Experiments with apple twigs

In the work reported here the twigs were gathered during the first two weeks in March, 1926. Only the one-year-old wood was used. The cut ends were paraffined. The twigs were exposed to room temperature for only a few minutes. From 150 to 200 gm. of the twigs were packed into glass tubes about 5 cm. in diameter and about 45 cm. long, and the tubes placed in the thermostat immediately. The temperature was 0° C. in these

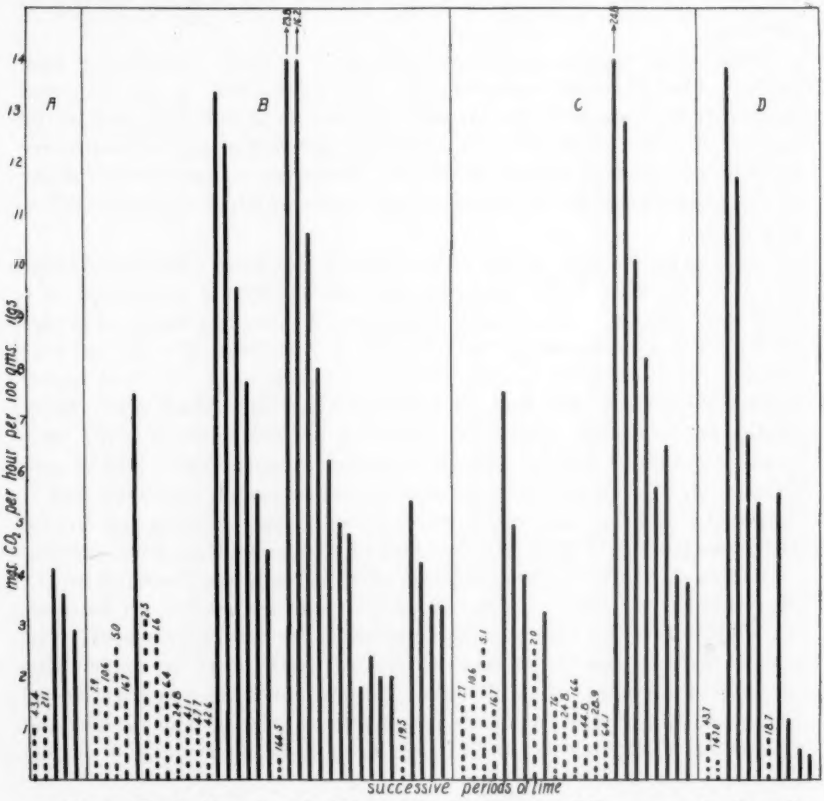

FIG. 1. Respiration of apple twigs at 0° C. The dotted line represents the rate during a period when the CO_2 is allowed to accumulate; the solid line represents a 20-minute period of continuous removal of CO_2. A, B, C, and D are different lots of twigs.

experiments. The thermostat, controls, air washing and circulating devices, and absorption towers were the same as described elsewhere (5).

The data are presented in figure 1. The vertical axis represents the rate of CO_2 production, expressed as mg. of CO_2 per hour per 100 gm. of twigs. The horizontal axis represents successive periods of time. The dotted lines stand for periods during which the CO_2 was allowed to accumulate, followed by a 20-minute period of aspiration to remove the CO_2. The lengths of these periods in hours is indicated by the number at the end of the dotted line. The solid line represents a 30-minute period, 20 minutes of which were consumed in aspiration, and 10 minutes in changing apparatus in preparation for the next period of aspiration. These half-hour periods thus represent periods of practically continuous aspiration. The time required for complete removal of CO_2 in the chamber, was, of course, carefully determined, and found to be about 12 minutes; 20 minutes were then adopted for safety. The object of this schedule was to measure the rate of CO_2 emission during varying periods by the discontinuous principle, followed by its measurement with continuous aspiration. Sections A to D represent different lots of twigs.

It will be seen that each dotted line or each series of them, is followed by a series of solid lines, each succeeding one of the latter being shorter. This means that a period in which the CO_2 is allowed to accumulate in the chamber is followed immediately by a much higher rate of respiration. The rate gradually subsides, but in the one case in lot B where a constant rate was attained, it was after about 35 hours.

There is some evidence that the magnitude of this phenomenon is proportional to the amount of CO_2 that has accumulated in the chamber, or in other words, the length of time of this accumulation. In lot C it is slightly evident after a 2-hour period. It is most pronounced following the 164.5 hour run in B and the 64.1 hour run in C. Seven periods of moderate length in B are as effective as two periods of much greater length in D.

It is quite evident that from these data, it would be impossible to say what is the normal rate of respiration of these twigs. Continuous removal of the CO_2 is imperative.

Casual study of the data for the accumulation periods indicates that in general the rate for the period is inversely proportional to the length of the period: in other words, that the rate continuously decreases with time. In order to bring out this relation more clearly, the amount of CO_2 for each period was plotted against the length of the period. The resultant graph is shown in figure 2.

A curve was fitted to these points. This curve corresponds to a logarithmic one expressed by the formula:

$$\frac{CO_2}{\log t - 0.566} = k$$

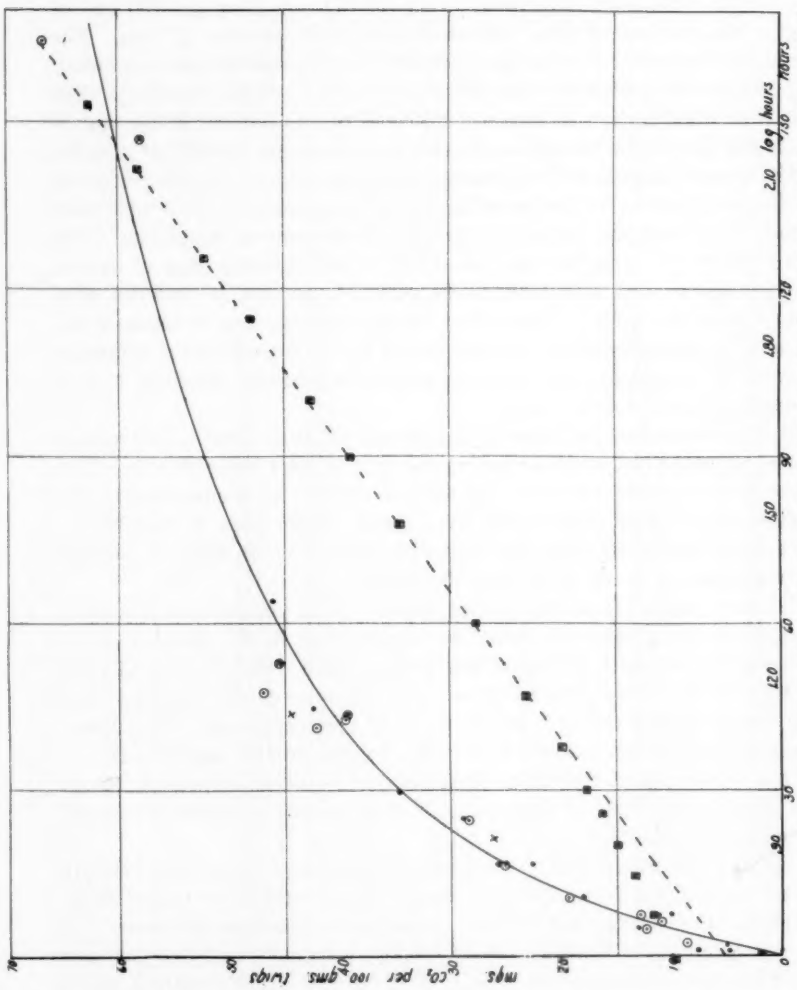

FIG. 2. Respiration of apple twigs at 0° C. as determined by the accumulation method. The symbols along the solid line represent different lots of twigs, with the CO_2 plotted against time in hours. The dotted line represents CO_2 plotted against log of time, using points on the solid line.

The solid line is CO_2 against t in hours, while the dotted line is CO_2 against log t. Table I gives the values of k for certain values of t.

TABLE I

VALUES OF k FOR THE RESPIRATION OF APPLE TWIGS ACCORDING TO THE CURVE IN FIGURE 2

t	CO_2	$\frac{CO_2}{\log t - 0.566}$
hours	mg.	k
5	10.0	75.2
10	17.7	40.8
20	28.0	38.1
30	34.6	38.0
40	39.3	37.9
50	42.7	37.7
70	48.2	37.7
90	52.2	37.8
100	53.9	37.6
130	58.3	37.7
170	62.7	37.7

The curve is in satisfactory agreement with the experimental values. The log-time curve is a straight line beyond the 30- or 40-hour period. During the shorter periods it is not a straight line because the respiration values are affected by the high temporary rates at the beginning of each period, in accordance with the data of figure 1 previously discussed. If the short period measurements had followed in all cases periods of continuous aspiration, during which the rate of CO_2 emission had attained a constant value, these short period measurements would no doubt have conformed much more closely to the equation.

This fact emphasizes the necessity, in plant respiration work, of taking cognizance of the history of the material during the period immediately preceding that of the measurement.

Experiments with potato tubers

Small potato tubers which would just enter the respiration tube were used in the same type of experiments. The tube was kept on the laboratory table at room temperature without special control. Any change in temperature during the continuous phase of aspiration was guarded against, and this is the only place that small changes in temperature could change the present results.

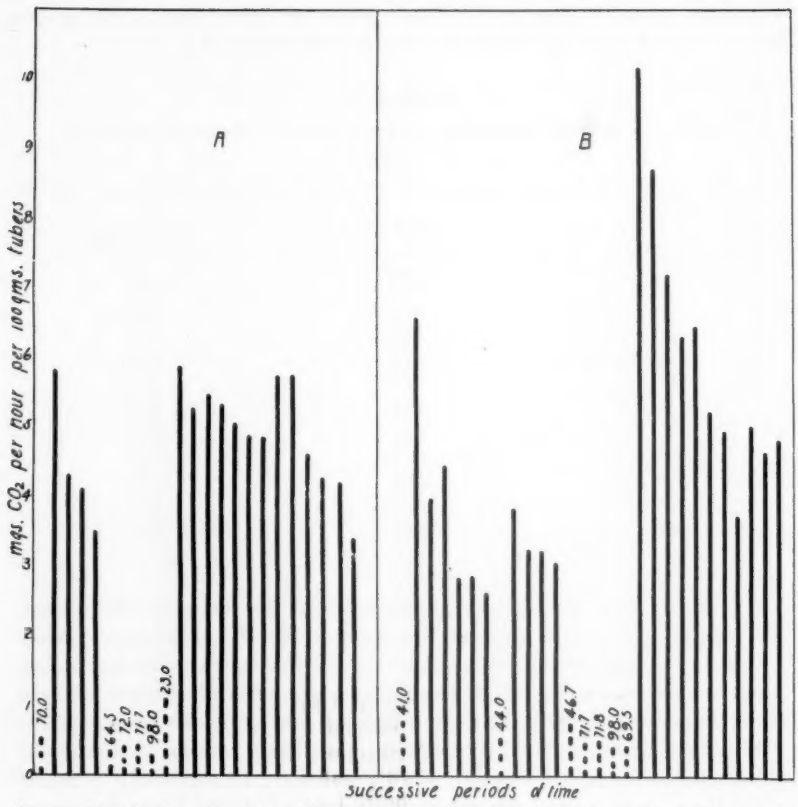

FIG. 3. Respiration of potato tubers at room temperature. See fig. 1 for description of conventions.

The results with two lots of tubers are shown in figure 3. The effect of accumulated CO_2 on the subsequent rate of its production is practically the same as in the case of the twigs.

Experiments with wheat

Since the results of BAILEY and GURJAR (3) indicate that the respiration of wheat grain may be affected by accumulated CO_2 in a manner similar to apple twigs, a series of experiments was run to determine this effect. Sound Marquis wheat was tempered to contain about 16 per cent. moisture. Respiration tubes like the ones used for twigs were filled with the wheat, about

500 gm. being required. The tubes were placed in a water thermostat at 40° C. This relatively high temperature was used because of the much slower rate of CO_2 production of this material in comparison with twigs.

The results are presented in figures 3, 4 and 5. In these runs the 20

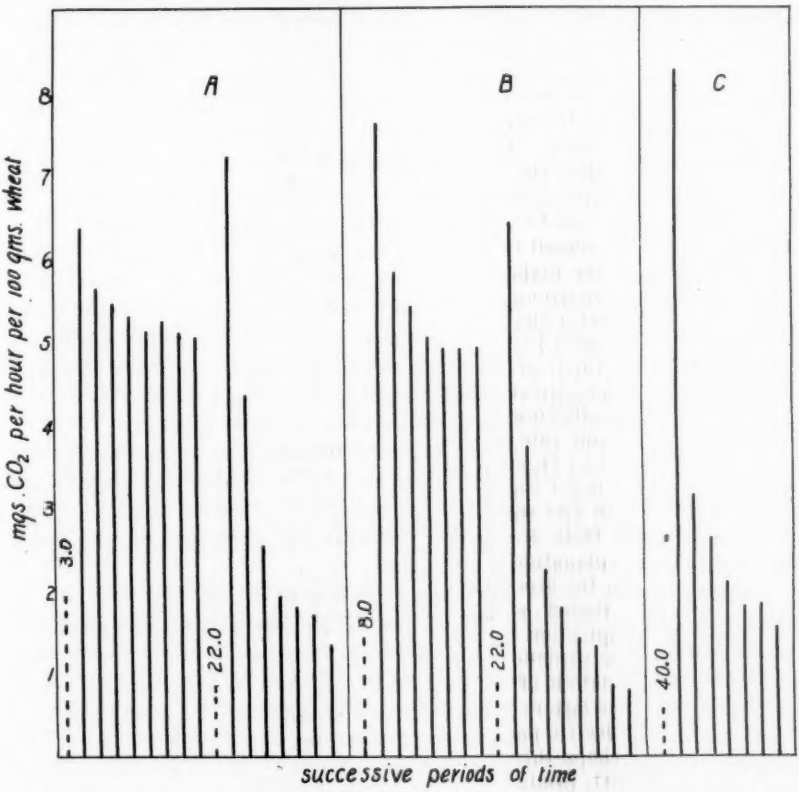

FIG. 4. Respiration of wheat at 40° C. See fig. 1 for description of conventions.

minutes of aspiration was followed immediately by a second aspiration. Therefore the solid lines represent 20-minute periods instead of 30. Practically the same picture is obtained here as in the case of the twigs and of the tubers. Each period of accumulation is followed immediately by an increased rate of CO_2 production. The rate gradually subsides, probably becoming constant after two or three hours, although this was not attained in any of these runs.

A possible explanation of the CO_2 effect

It has been shown that three types of plant tissue, twigs, tubers and wheat grain, exhibit the same reaction to the accumulation of CO_2 in the surrounding atmosphere. For the sake of clearness, this reaction may be described again: When the CO_2 is allowed to accumulate, the rate of production of the CO_2 diminishes in a logarithmic ratio. In the case of twigs, at least, the amount of CO_2 produced is proportional to the logarithm of time. When accumulated CO_2 is removed, the rate of its production immediately assumes a far higher value; and the magnitude of this increased value is possibly proportional to the amount of CO_2 previously accumulated. It is a matter of several hours before the rate attains a constant value.

This is in strict accord with the conclusions of SPOEHR and MCGEE (**23**): "When the CO_2-content of the air surrounding a leaf is changed from a lower to a higher concentration, the leaf shows a reduced rate of CO_2 emission for a period following the change, then increases, and finally again attains about the same rate as before the change in CO_2-content was made. Conversely, when the CO_2 content of the air surrounding a leaf is changed from a higher to a lower concentration, the leaf shows a primary increased rate of CO_2-emission and subsequent decrease to the original rate."

Such being the facts, we are of course interested in attempting an explanation. One explanation is that we are observing merely an equilibrium between the CO_2 in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess CO_2 in the latter is removed but slowly when aspiration is commenced.

Another possible explanation was suggested by Dr. R. A. GORTNER. It is that the accumulation of CO_2 in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point, and hence increases its permeability, which is responsible (perhaps through increased enzyme activity) for an actual increased rate of CO_2 production. The increase would probably be merely potential as long as there was a high content of CO_2 in the air surrounding the tissues, but would become actual as soon as aspiration was commenced.

It will be necessary to review the existing evidence in favor of such a proposition before presenting the experiments designed to demonstrate it.

That the membranes would be more permeable if their proteins were nearer the isoelectric point is possibly illustrated by the experiments of HITCHCOCK (**13**). We can best quote from him directly: "The permeability of gelatin-coated collodion membranes, as measured by the flow of water or of dilute solutions through the membranes, has been found to vary with the pH of the solutions. The permeability is greatest near the isoelectric point of the protein; with increasing concentration of either acid or alkali it

decreases, passes through a minimum, and then increases. These variations with pH are qualitatively in accord with the assumption that they are due to swelling of the gelatin in the pores of the membrane, the effects of pH being similar to those observed by LOEB on the swelling of gelatin granules. Indications have been found of a similar variable permeability in the case of membranes coated with egg albumin, edestin, serum euglobulin, and serum albumin."

That the acidity of the tissue fluids is increased by the accumulation of CO_2 is well known, and does not need a specific illustration. That such an increase in acidity would bring the proteins nearer to their isoelectric points is, however, not so easy to argue from existing data. The isoelectric point of but few plant proteins has been determined. CHIBNALL (**8, 9, 10, 11**) has isolated glutelins from the leaves of spinach, corn, alfalfa and other plants, and has found them to have very similar properties, with an isoelectric zone of pH 4.0 to 5.0. In all cases the reaction of the expressed sap was alkaline with respect to the isoelectric point of the protein. The gliadin of the wheat berry has an isoelectric point of 6.6 according to ETO, and 5.76 according to HOFFMAN and GORTNER (**2,** p. 245); that of the glutenin is 6.8 to 7.0 (**2,** p. 250); and that of the leucosin is 4.6 (**2,** p. 253). ROBBINS (**20, 21, 22**) has determined what he believes to be the isoelectric point for tissues, and has obtained the following values: potato tuber, 6.0 to 6.4; mycelium of *Rhizopus nigricans,* 5.0; that of *Fusarium lycopersici,* 5.5; that of *F. oxysporum,* 4.9; that of *Gibberella saubinetii,* 6.2; soy bean root tips, 6.2 to 6.4.

The most careful measurements of the pH of cell sap are those by NEEDHAM and NEEDHAM (**17**) and by CHAMBERS and POLLACK (**7**), in which experiments indicator dyes are introduced into the cell by micrurgical technique. The former obtained values of 6.6 for various marine ova, and the latter 6.6 to 6.8 for starfish eggs. When the cell is injured, as by a tear, the pH rapidly diminishes to pH 5.4 to 5.6. If the latter phenomenon holds also for plant cells, the conclusion is that the host of measurements of the pH of expressed sap indicate a higher acidity than actually obtains in the normal tissue. Most of such expressed saps show a pH of 5.0 to 7.0.

Taking a general survey of the data reviewed above, it is fair to conclude that there is some indication that plant sap is alkaline with respect to the isoelectric point of its proteins. CHIBNALL has the only direct evidence of this. If this relation be assumed, the conclusions would follow that CO_2 could bring the reaction nearer to the isoelectric point of the proteins, that this would increase the permeability of the protoplasm, and that more rapid respiration could result.

A method used in the attempt to obtain more or less direct evidence of this chain of events was to introduce HCl gas, for short periods, into the

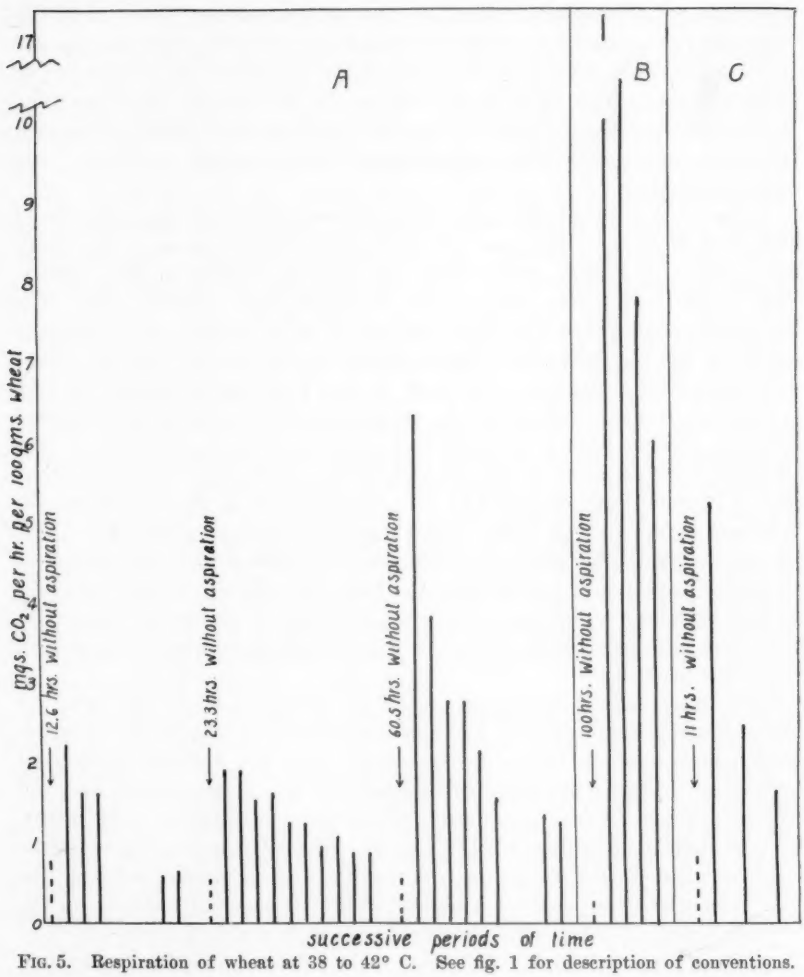

FIG. 5. Respiration of wheat at 38 to 42° C. See fig. 1 for description of conventions.

respiration chamber containing wheat and to observe whether this increased the rate of emission of CO_2. This has been done, with fairly positive results in favor of the above hypothesis.

The air entering the chamber was first bubbled through a solution of HCl for 30 minutes in one run, and for 1.5 hours in the other. The chamber then remained for one hour without aspiration. Then the aspiration was continued and the CO_2 determined. The titration procedure was modified

so as to allow for any HCl that might accompany the CO_2. HNO_3 was used for titrating instead of the usual HCl; $Ba(NO_3)_2$ was used to precipitate the carbonates instead of $BaCl_2$; and at the end of the acid titration the chlorides were titrated with silver nitrate. Only a few mg. of HCl reached the absorption tower.

The results of the two experiments are given in figure 6. In the trial run shown in the upper portion of the graph, aspiration was continued for one hour so as to establish the rate of CO_2 production. Then the air was bubbled through concentrated HCl for 30 minutes. After standing for one hour, the accumulated CO_2 was measured, and is represented by the dotted line in C. Following this there is seen to be the usual increase in rate, followed by a decrease to a level which is somewhat lower than the rate in A. Another one-hour period of standing (D) was followed by another but slighter increase.

In the second run, in the lower half of figure 5, a control period without HCl was measured first. This involved a cycle of three periods (A, B, C). Following this was the treatment with HCl as indicated in the chart, and the measurement of the subsequent rate of CO_2 production. Period C, following the control, is characterized by an initial rate of 3.5 mg. Period F, following the HCl treatment, is characterized by an initial rate of 5.4 mg.

The writers are convinced that the HCl treatment has duplicated the effects of accumulated CO_2. To be sure, the wheat was sooner or later injured by the treatment, but it was not visible during the course of the runs, and in any event the increase in CO_2 production is a fact.

Another line of evidence that disfavors the idea of mere solution of CO_2 in the tissues is the size of the three tissues used. It is reasonable to expect that the thicker the tissue, the slower will be the rate of diffusion of the dissolved CO_2 into the surrounding air. In the three tissues used the time required to attain a constant value should decrease in the order, wheat, twigs, tubers. There is no evidence of this differential rate in the charts.

The writers do not believe that the exhaustion of oxygen from the chamber is a deciding factor. In the case of the twigs and the tubers, the oxygen was never exhausted; in the case of the wheat it was exhausted only during a few long runs. Furthermore KARLSON (**14**) states: "The effects of ether, benzene, and alcohol on the aerobic and anaerobic production of CO_2 by wheat (seedlings) are closely similar. This would seem to indicate that the fundamental processes or the master reactions on which they depend are similar."

On the basis of these experimental findings and of the suggestive evidence of the pH values found in the literature, the writers are led to adopt

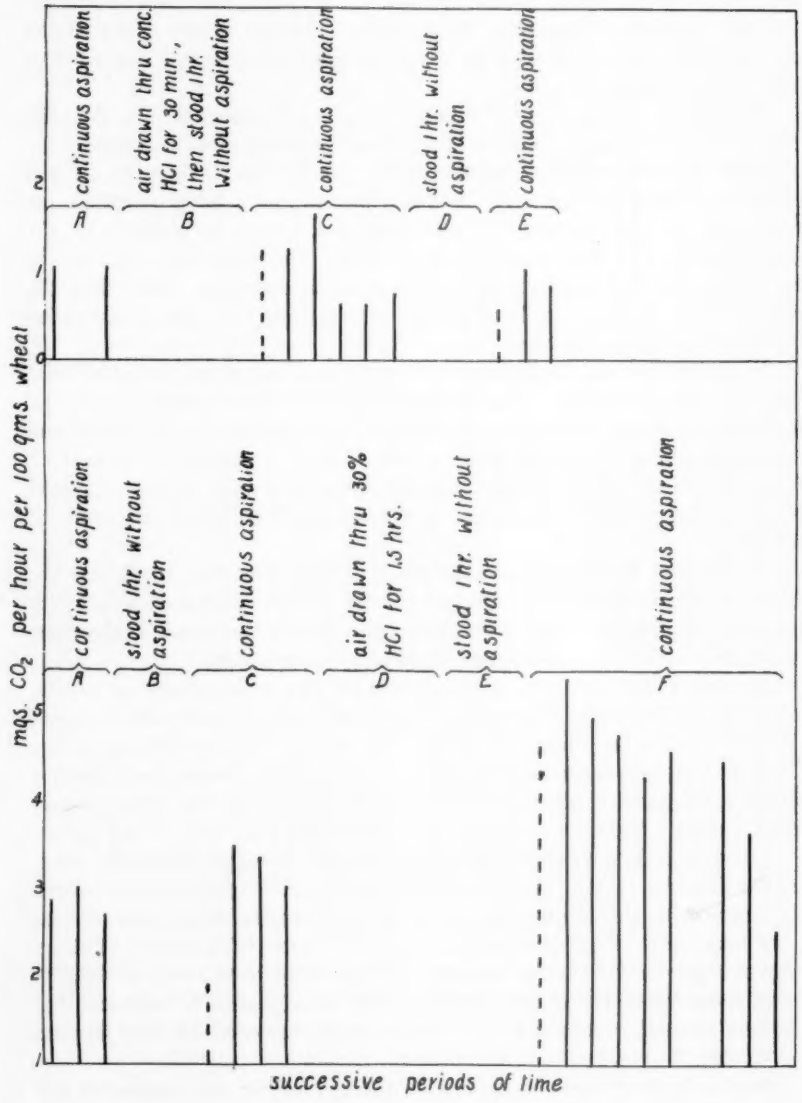
mgs. CO_2 per hour per 100 gms. wheat
successive periods of time
A continuous aspiration
B stood 1hr. without aspiration
C continuous aspiration
D air drawn thru 30% HCl for 1.5 hrs.
E stood 1 hr. without aspiration
F continuous aspiration
A continuous aspiration
B air drawn thru conc. HCl for 30 min., then stood 1hr. without aspiration
C continuous aspiration
D stood 1 hr. without aspiration
E continuous aspiration

FIG. 6. Respiration of wheat at 40° C. as affected by HCl gas.

the explanation here outlined for the effect of accumulated CO_2 on the rate of respiration of plant tissues.

Conclusions

The respiration of apple twigs at 0° C., of potato tubers at about 22° C., and of wheat grain at 40° C. has been studied from the standpoint of the effect of allowing the CO_2 to accumulate in the respiration chamber. Under such conditions the rate of CO_2 production continuously decreases with time. After the first 30 or 40 hours the relation is expressed by the formula

$$\frac{CO_2}{\log t - 0.566} = k.$$

During the first 30 or 40 hours the rate is affected by a phenomenon which can be described as follows: When aspiration of the atmosphere surrounding the tissue is commenced, after a period of accumulation of CO_2, the rate of respiration immediately assumes a far higher value than it had during the accumulation period. The magnitude of this value is possibly proportional to the amount of CO_2 previously accumulated. It is a matter of several hours before the rate attains a constant value.

One explanation of this phenomenon is that we are merely observing an equilibrium between the CO_2 in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess CO_2 in the latter is removed but slowly when aspiration is commenced.

Another possible explanation is that the accumulation of CO_2 in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point and hence increases the permeability of the protoplasm; and that this is responsible for an actual increase in rate of CO_2 production. The evidence in the literature on the pH of cell sap and on the isoelectric points of plant proteins bears out this view to a certain extent. Direct evidence in its favor was secured by passing HCl gas into a respiration chamber containing wheat grain. A duplicate of the CO_2 effect was obtained.

Although admitting that the proof for the latter explanation is still far from complete, the writers nevertheless subscribe to it, and offer it for the criticism of others.

The conclusion should be emphasized that the investigator should take cognizance of the CO_2 effect in deciding which procedure, the continuous or the discontinuous, to adopt for the work in hand. Under some circumstances, of course, as in a study of grain and fruit in storage, more useful knowledge might be secured by the accumulation method.

The writers wish to acknowledge the help of Mr. Leo M. Greene in securing some of the data in this paper.

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ON THE NITROGEN CONTENT OF GROWING CULTURES OF *MYCODERMA* AND OF *SACCHAROMYCES CEREVISIAE*

LEO M. CHRISTENSEN

Introduction

In a previous communication by FULMER and CHRISTENSEN (5) it has been shown that the nitrogen content of a growing culture of an organism designated as "yeast no. 12" is a function of time and of pH as determined by the Kjeldahl method. It was also noted that the cultures showed a preliminary loss in nitrogen followed by a gain and that the greatest gain took place at the pH at which there was the antecedent maximum loss. It was suggested that the loss may have been more apparent than real, the hypothesis being that the nitrogenous compounds may have undergone a change rendering them unanalyzable by the regular Kjeldahl method. This led to the development of a modified method, utilizing a preliminary oxidation with hydrogen peroxide in dilute sulphuric acid solution. This method which gives higher yields of nitrogen for yeast than the usual Kjeldahl method has been described in detail by CHRISTENSEN and FULMER (2).

The data presented in this communication deal with the change in nitrogen content of growing cultures of the organism previously studied and of *Saccharomyces cerevisiae* as determined by the two analytical methods, the Kjeldahl and the modified method previously mentioned.

Organisms used

Two types of organisms have been used in our studies of yeast nutrition, both of which were isolated from a cake of Fleischmann yeast and designated by us as yeasts nos. 11* and 12* (1). These two types resemble those described by EDDY, KERR and WILLIAMS (3). Number 11 is *Saccharomyces cerevisiae*. On three different occasions within the course of a year no. 12 was found to compose about 2 per cent. of the organisms in the commercial product. This type was isolated from the same source by MACDONALD (7) and classified as a *Mycoderma* by this author. A careful study of the organism in this laboratory verifies this classification.

The properties of the two organisms are given in table I.

* These organisms are listed in the American type culture collection as no. 4226 (*Saccharomyces cerevisiae*) and no. 4225 (*Mycoderma*) respectively.

TABLE I

PROPERTIES OF ORGANISMS COMPARED IN VARIOUS MEDIA

PROPERTIES OBSERVED	SACCHAROMYCES CEREVISIAE	YEAST NO. 12
Colonies on 2 per cent. glucose, 0.5 per cent. peptone agar		
Growth	Slow	Rapid
Form of Colony	Circular	Circular
Surface	Smooth, moist	Rough, dry
Elevation	Convex	Convex
Edge	Entire	Filamentous
Internal structure	Amorphous	Granular
Branching	Absent	Predominant
Shape and size of cells	Spherical or ellipsoidal, 6–8 μ diam.	Elongated, 8–20 μ x 2–4 μ
Size of colony 48 hours	1 mm. diameter	4 mm. diameter
Growth in medium E,* inoculated from colony on glucose-peptone-agar		
48 hours	Growth	Visible growth
72 hours	Count 89.5	Count 1.0
	Cells spherical, little branching	Cells spherical, little branching
Growth in medium K,† inoculated from colony on glucose-peptone-agar		
48 hours	No growth	Visible growth
72 hours	No growth	Count 23.
Fermentation of carbohydrates. (Peptone 0.5 per cent., carbohydrate 2.0 per cent.)		
Glucose	Gas and alcohol	Gas
Levulose	Gas and alcohol	Gas
Sucrose	Gas and alcohol	Small amount of gas
Maltose	Gas and alcohol	Gas
		Esters rather than alcohol are formed
Growth in wort		
24 hours	Foam	Heavy froth
48 hours	Count 620	Count 3380
	Bottom growth, cells spherical, 6–8 μ diameter, no branching	Dry, wrinkled surface growth. Cells elongated, 5 x 7 μ, branching predominant
Spore formation 10 day growth on carrot infusion-$CaSO_4$-agar		
	Typical ascospores of *S. cerevisiae*	No spores

* Medium E contained per 100 cc. 0.10 g. K_2HPO_4; 0.10 g. $CaCl_2$; 0.04 g. $CaCo_3$; 0.188 g. NH_4Cl; 2 g. sucrose after FULMER, NELSON and SHERWOOD (6).

† Medium K contained per 100 cc. of medium 0.10 g. K_2HPO_4; 0.002 g. NH_4Cl; 2 g. sucrose.

It is apparent that the *Mycoderma* grows more poorly in medium E than the yeast, while the reverse is true for the medium low in nitrogen. Several organisms kindly furnished by F. W. TANNER were grown on medium K. All of these organisms except *Saccharomyces cerevisiae* had a tendency to grow on the surface, a characteristic which would seem desirable under conditions in which nitrogen is taken from the air. The organisms so tested and the summary of results are listed in table II.

TABLE II

GROWTH ON MEDIUM K OF SEVERAL ORGANISMS

ORGANISM	GROWTH
No. 12 (*Mycoderma*)	++++
Willia saturnus	++++
Torula humicola	+++
Willia anomala	++
Saccharomyces chevalieri	++
Mycoderma vini	++
Tichia membrane faciam	+
Saccharomyces anomalus	+
Saccharomyces neoformans	+
Saccharomyces hominis	+
Saccharomyces cerevisiae	+

There is evidently a considerable difference in the ability of these organisms to grow in a low nitrogen environment. In the following studies the first (*Mycoderma*) and last organisms (*Saccharomyces cerevisiae*) were employed.

The media employed

In omitting the ammonium chloride from the medium, not only was the organism deprived of a good source of nitrogen but likewise of the physico-chemical effect of the ammonium chloride. This rôle has been emphasized by FULMER and co-workers (**4, 6**). The growth of the *Mycoderma* in the synthetic media tested was not large enough to permit consistent quantitative estimation of changes in nitrogen content of the medium. It seemed advisable then to add the growth stimulant, bios, in amounts permitting sufficient growth for analysis and with the minimum addition of nitrogen. For this purpose high-grade molasses, one sample containing 0.21 per cent. nitrogen (Kjeldahl) and the other 0.41 per cent. nitrogen, was used.

The medium contained per 100 cc. 6 g. molasses and 0.50 g. K_2HPO_4. The pH of the medium was adjusted after sterilization by the method and apparatus described by CHRISTENSEN and FULMER (**1**).

Experimental method

The incubator in which the organisms were grown was placed in a room which for several years had been used for water analysis and hence was unusually free from contamination with ammonia or nitric acid fumes. A current of air passed through potassium permanganate, sodium hydroxide, and sulphuric acid solutions was passed through the incubator.

Each analysis was run on a separate flask, several hundred of which were required for the experiments described. All flasks including the blanks were inoculated to a count of one (250,000 cells per cc.), a blank being used for each pH value. The flasks designed for blanks were sterilized with live steam in order to kill the yeast.

The fact that nitrogenous compounds were not taken from the air in a period of 6–8 weeks through the pH range used is shown by the data in table III.

TABLE III

NITROGEN CONTENT OF BLANKS (MG./100 CC.)

EXPERIMENT	A	B	C						
TIME (WEEKS)	6	6	1		2		4		6
METHOD	I	I	I	II	I	II	I	II	I
pH									
4.0	12.70	12.51	29.48	39.19	30.94	42.74	29.41	40.99	.....
5.0	12.60	13.18	29.41	42.09	30.30	41.81	29.83	41.66	29.41
6.0	12.80	12.20	29.41	40.39	31.25	42.37	30.12	41.32	.....
7.0	.....	12.20	29.24	39.56	30.30	41.81	30.30	41.32	29.76
8.0	12.70	12.82	29.59	40.42	30.12	41.32	30.30	40.42	29.94
9.0	12.70	12.20	29.41	42.09	29.83	.....	30.49	41.53	29.76
Average	12.70	12.53	29.42	40.62	30.46	42.01	30.09	41.21	29.72

Mean I = 30.08; II = 41.25.

Experiments A and B were run with molasses containing 0.20 per cent. N (Kjeldahl) and C with molasses containing 0.41 per cent. N (Kjeldahl).

I and II refer to the Kjeldahl and modified Kjeldahl respectively.

Two methods of analysis were used: I. The regular Kjeldahl method. II. The modified method. This involves the addition of hydrogen peroxide to 15 per cent. and 0.1 per cent. sulphuric acid as previously described by CHRISTENSEN and FULMER (2). The mixture was evaporated nearly to dryness over a slow flame. After the residue was cooled the regular Kjeldahl procedure was employed.

The ammonia was determined by Nesslerization.

Experiments with *Mycoderma*

The results of the experiments with *Mycoderma* are given in table IV. The values for the blanks have been previously given in table III.

TABLE IV

ANALYSIS OF NITROGEN IN CULTURES OF *Mycoderma* (MG./100 CC.)

EXPERIMENT	A						B*				
TIME (WEEKS)	1	2	3	4	6	8	1	2	3	6	8
METHOD	I	I	I	I	I	I	I	I	I	I	I
pH											
4.00						−0.9					−0.8
4.50						−1.5					+0.4
5.00					−4.5	−2.1					+0.6
5.25					.....	.....					.....
5.50					−1.9	−1.2					+0.9
5.75					.....	.....					.....
6.00					.....	−0.5					+0.5
6.20					−0.5	.....					.....
6.25					.....	.....					.....
6.40					−1.3	.....					.....
6.50					.....	−2.1					+0.2
6.75					.....	.....					.....
6.80					−3.2	.....					.....
7.00					−2.5	−2.1					−1.1
7.20	−3.6	−3.4	−4.7	−4.2	−1.9	−0.9	−5.7	−4.2	−4.0	+2.6	+0.4
7.25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.40	−2.6	−3.5	−2.6	−2.5	+1.2	−0.7	−5.1	−2.9	−2.7	+1.2	+0.2
7.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.60	−3.2	−4.5	−3.6	−3.4	+2.0	+1.3	−4.7	−3.8	−4.6	.....	±0.0
7.75	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.80	−1.0	−1.2	−5.3	−3.6	+2.3	+3.8	−4.4	−6.9	−4.5	+0.2	+0.2
8.00	−0.9	−3.2	−7.0	−3.7	+1.6	+4.9	−3.5	−6.1	−4.1	+0.2	+0.4
8.25					.....	.....					.....
8.50					−0.8	+3.3					+0.2
9.00					−1.8	+2.4					−1.7
9.50						+2.0					−1.0

pH and pH′ represent the hydrogen-ion concentration before inoculation, and after growth had taken place, respectively.

* This medium contained 10 g. sucrose per 100 cc. in addition to the molasses.

It will be noted that the results in experiment C are more erratic than those in experiment A. The latter experiment was run on a molasses con-

TABLE IV—(*Continued*)

ANALYSIS OF NITROGEN IN CULTURES OF *Mycoderma* (MG./100 CC.)

EXPERIMENT	C										
TIME (WEEKS)	1				2				4		6
METHOD			I	II			I	II	I	II	I
pH	pH′	Count / 10	I	II	pH′	Count / 10					
4.00	5.02	2.6	−1.0	−3.0	5.36	10.8	−0.3	+ 3.3	+3.2	−2.2	−5.1
4.50	5.71	2.2	−1.9	−3.1	6.11	9.5	−0.8	+ 9.0	+1.1	+1.1	−1.4
5.00	6.38	1.3	−3.5	.....	6.34	10.2	+0.9	+12.2	±0.0	+2.3	.....
5.25	6.05	2.2	−0.6	−2.8	6.39	12.3	+3.3	+ 2.0	+1.2	+3.5	+0.4
5.50	6.00	2.9	+0.7	−2.1	6.34	11.0	+1.3	+ 0.7	+1.6	+1.3	−3.2
5.75	6.28	2.5	+1.0	+6.9	6.54	13.5	+1.1	+ 6.6	+2.4	+3.4	.....
6.00	6.30	2.9	−0.3	+7.0	6.52	10.4	.....	+ 6.6	+0.7	+3.5	.....
6.20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6.25	6.85	1.3	−0.7	+8.7	7.03	9.4	+3.1	+ 6.1	+2.0	+0.5	.....
6.40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6.50	.....	.....	+0.1	+5.5	7.27	8.8	+1.0	+ 8.7	−0.6	−0.3	−2.3
6.75	7.22	1.9	.....	.....	7.69	5.8	+1.3	+ 0.6	+0.4	−1.2	−0.5
6.80	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.00	7.31	1.9	−0.5	+4.3	7.69	7.3	−1.5	+ 9.3	+1.0	+0.1	−4.2
7.20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.25	7.35	1.2	±0.0	−7.5	7.75	8.2	−1.4	+ 1.5	+2.2	+1.5	.....
7.40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.50	7.48	1.8	−0.5	−9.0	8.10	7.7	−1.4	.....	.....	+0.4	−2.6
7.60	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.75	7.67	1.9	−3.5	−2.7	8.12	7.3	+0.3	+ 6.4	.....	+3.06	.....
7.80	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8.00	7.77	1.5	.....	−2.3	8.31	8.7	+1.2	+ 1.1	−0.6	−2.8	−2.4
8.25	7.83	1.0	−3.4	−1.2	8.37	8.6	+3.3	+ 1.0	+0.3	−1.0	−3.5
8.50	7.91	.....	−2.4	−1.2	8.55	8.2	−1.1	+ 5.0	+1.6	+2.7	+1.6
9.00	8.54	.....	−1.0		8.74	6.1	−2.3	+ 6.1	+1.6	.....	
9.50											

taining about one-half the nitrogen in the former. This may account in part for the irregular results. The larger the amount of nitrogen present the greater is the probability of error in dealing with small differences. From the data certain conclusions may be drawn.

1. The amount of nitrogen in the culture is a function of pH and time.

2. The modified Kjeldahl method shows much greater gains as well as greater losses than the regular Kjeldahl method. In our previous communication we had suggested that the fact that the pH in which there was the greatest preliminary loss in nitrogen coincided with the final greatest

gain, was due to analytical method. Evidently the new method does not solve the difficulty.

3. Whether a gain or loss of nitrogen is found depends upon the method used for analysis. Conditions determined to be optimal by one method of analysis may not appear optimal when another analytical method is used.

4. After a long period the cultures all begin to lose nitrogen as determined by both methods, the loss being relatively greater with the modified than with the Kjeldahl method.

5. There is no obvious correlation between the number of cells and the phenomena discussed above.

6. The pH of the medium changes in such a way that the alkaline become more acid and the acid more alkaline narrowing the pH range of the series from a range of about 5.5 units initially to about 3.5 in one week and to 3.4 in two weeks.

Experiments with *Saccharomyces cerevisiae*

The results for *Saccharomyces cerevisiae* are given in table V.

The results are erratic due to the small changes involved, but the general tendencies are the same as those for *Mycoderma*. The modified method

TABLE V

ANALYSIS OF NITROGEN IN CULTURES OF *Saccharomyces cerevisiae* (MG./100 CC.)

TIME (WEEKS)	1				2				4		6
pH	pH′	Count	I	II	pH′	Count	I	II	I	II	I
4.00	4.00	310	−0.33	−5.6	4.45	294	−2.1	−0.3	+0.7	−0.9	.....
4.50	4.60	380	−0.49	−5.9	4.68	303	−1.4	−1.6	−0.3	+4.0	−3.1
5.00	5.00	345	−0.33	−6.1	4.85	304	+2.5	−2.5	+0.3	+1.5	−3.8
5.25		325	−0.49	−5.5	4.96	304	+0.2	−2.8	−1.3	+4.9	+0.8
5.50		360	−0.33	−5.8	5.06	348	+1.9	.....	+1.5	+4.1	−2.70
5.75		355	−0.49	−6.1	5.15	348	+0.0	.....	+0.4	+0.4	−1.5
6.00	5.20	415	−1.3	−5.8	5.74	348	−3.3	−2.8	−2.7	+1.2	.....
6.25		390	−1.3	−6.1	5.80	386	+0.4	.....	+0.2	+1.8	−1.5
6.50		400	−1.3	−5.3	6.08	382	+1.2	.....	+1.5	+5.5	.....
6.75		370	−1.5	−5.5	6.11	408	−1.2	.....	.....	−3.3	.....
7.00	6.40	334	−2.2	−5.5	6.14	394	−0.7	−1.3	+2.1	−4.4	.....
7.25		360	−2.6	−6.4	6.20	376	−0.3	−0.8	+1.1	+1.3	−1.5
7.50		370	−3.1	−6.1	.....	.....	−0.7	−1.3	−0.5	−3.3	.....
7.75		445	−2.6	−6.5	.....	.....	−2.1	−1.6	+0.5	.....	.....
8.00		.....	−2.3	−6.1	6.24	372	−1.3	.....	+0.0	+0.5	−5.9
8.25	6.60	350	.....	−5.5	6.28	393	−0.7	−0.8	+1.2	−0.3	−6.0
8.50		360	−2.3	−5.5	6.43	342	−1.2	.....	+2.0	+1.1	−0.2
9.00	6.80	285	−0.86	−5.5	6.62	301	+0.8	.....	+0.2	+0.5	.....

again shows that the greatest losses and the greatest gains are both functions of pH. There is the same preliminary loss followed by gain and with a subsequent loss after six weeks. The striking difference is in the pH change which is much greater with the *Saccharomyces*. An original range of 5 pH units is in two weeks narrowed down to 2.2 units. This means that the pH range finally involved is less than for the *Mycoderma*. There is no obvious correlation between the number of cells and the losses or gains in nitrogen. Through the pH range tested the changes in nitrogen content are much less than with the *Mycoderma*; however, as indicated above, the pH range is finally less than with the *Mycoderma*.

Summary

The nitrogen content of cultures of *Mycoderma* and of *Saccharomyces cerevisiae* in molasses at various values of pH has been followed by two methods of analysis, the regular Kjeldahl and a modified Kjeldahl previously described from this laboratory. The modified method magnifies both the losses and gains in nitrogen. The maximum losses and maximum gains are not in all cases at the same pH by both methods. Following the maximum gain there is a loss in nitrogen. The nitrogen content of the cultures is dependent then upon pH, time and method of analysis. The results indicate that neither method gives all the nitrogen at any given pH or time interval. It is evident that results on nitrogen fixation are more likely to err on the negative than on the positive, that is, there probably is more fixation than any available method indicates.

The author wishes to thank Dr. E. I. FULMER for suggesting the above problem and for aid throughout the work; he also appreciates the aid of E. E. MOORE in obtaining some of the analyses.

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THE DEVELOPMENT OF CHLOROPHYLL IN SEEDLINGS IN DIFFERENT RANGES OF WAVE LENGTHS OF LIGHT*

J. D. SAYRE

(WITH THREE FIGURES)

Introduction

While investigating the behavior of stomata in different kinds of light it was noticed that no chlorophyll developed in seedlings grown under some of the colored glass plates used in that work. The object of this investigation was to find out more fully, if possible, the wave lengths of light necessary for the development of chlorophyll in plants. A search of the literature yielded very little information on the subject. The only record of any other work of this kind is in PALLADIN'S Plant Physiology (**4**), where WIESNER used double walled bell jars with different colored solutions to separate the spectrum into different parts. He reported that chlorophyll was formed in both the red-orange-yellow region, produced by potassium dichromate, and also in the green-blue-violet region produced by copper sulphate. The non-luminous heat rays were not effective. There is plenty of information on the effects of various wave lengths of light on photosynthesis, especially on those wave lengths which produce maximum photosynthesis. This is very well summarized by SPOEHR (**6**).

Methods used

Seedlings were grown under colored glass plates and their color noted. No measurements of the amount of chlorophyll in the tissue were made. The relative greenness of the plants when compared with controls grown under all wave lengths of light was taken as a measure of the chlorophyll present. All plants were grown under similar temperature, humidity, and soil conditions, the variable being the different wave length of light. Seedlings of corn, wheat, oats, sunflower, radish, mustard, and bean were used in this work.

The colored glass plates used were the 6½ inch square ray filters manufactured by the Corning Glass Works, Corning, N. Y. The same series as used in the spectral glass houses of the Boyce Thompson Institute, reported by POPP (**5**), were employed with a few additions. The manufacturers'

* Paper from the Department of Botany, The Ohio State University, no. 210.

numbers of these plates are G86B, G38L, G38H, G34, G24, G124J, G584J, G55A, G585L, and G586A.

The transmission data for these glass plates were obtained from the Bureau of Standards Technologic Papers by COBLENTZ and EMERSON (**1**), GIBSON and MCNICHOLAS (**2**), and GIBSON, TYNDALL, and MCNICHOLAS (**3**). These data were converted by means of the chart and instructions given by GIBSON and MCNICHOLAS (**2**), values for the average thickness of the plates used. The values for combinations of two or more plates is the product of the data for each plate separately. All these data are given for an equal energy spectrum. The percentage of total transmission for each plate or each combination of plates was obtained by comparing the areas under the transmission curves when they were plotted on cross-section paper. These data were calculated for a spectrum extending from 300 mμ to 770 mμ, 770 mμ being the limit of visible spectrum as given by GIBSON and MCNICHOLAS (**2**). The transmission curves for these glass plates are shown in fig. 1 and fig 2. These data can be used only for plates of the thickness indicated in each case. The limits of transmission for each plate were checked by means of a wave length spectrometer loaned by the Physical Chemistry Department of the Ohio State University.

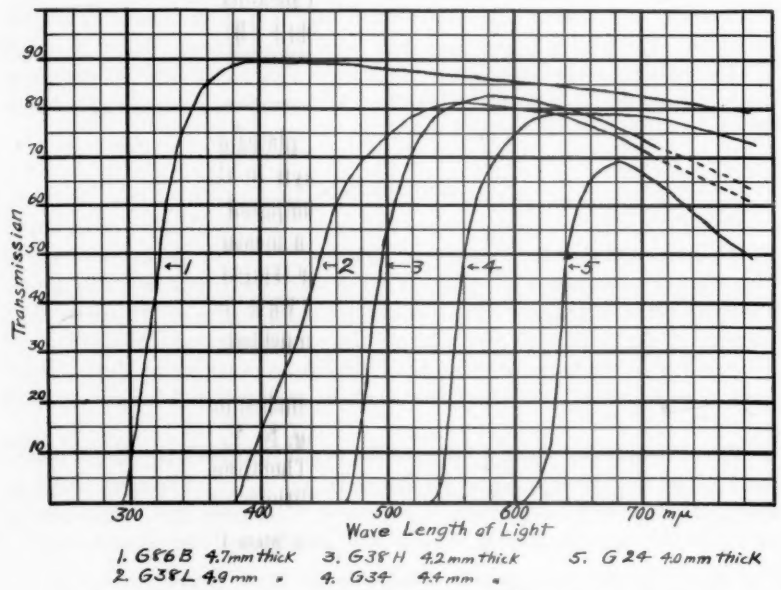

FIG. 1. Transmission curves of glass plates used in the experiments.

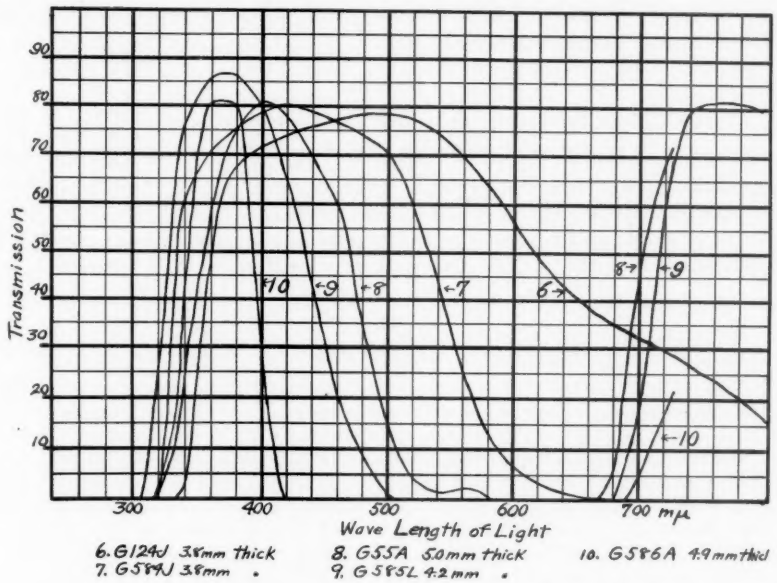

FIG. 2. Transmission curves of glass plates used in the experiments.

The data for the relative spectral distribution of radiant energy from the 400 watt Mazda C light were taken from GIBSON and MCNICHOLAS (**2**). The percentage of energy from the Mazda C light transmitted by each plate was found by calculation. The values of relative spectral distribution of energy from the Mazda C light were multiplied by the transmission value at each wave length and curves of these values plotted on paper. The areas under each curve were compared and reduced to percentages. These percentages are comparable when all plants are placed at equal distances from the light.

The plants were grown under ventilated, light-proof tin boxes which held four glass plates. The boxes were painted white (MgO paint) inside, and with aluminum paint on the outside. The seeds were planted directly in the soil when the experiments were conducted out of doors, and the tin boxes were sunk several inches in the soil to make a light-tight seal. In the greenhouse, flats of soil were used and the tin boxes were sunk for several inches into a large tray of soil. For the experiments under artificial light a large box was used. The plants were placed one meter from this light. Many experiments were tried, and many calculations of data were necessary for this work which has covered a period of several years at interrupted

intervals. The results of only two typical experiments are given since similar results were obtained each time.

Results

Table I gives the results of a series of experiments carried out in the greenhouse in the fall and winter of 1926–27. This series covered a period of six to seven weeks, since only five sets of plates and a control could be used at one time. The numbers of the glass plates are given with their limits of transmission, and the percentage of an equal energy spectrum transmitted by each. The development of chlorophyll is expressed by

TABLE I

TRANSMISSION DATA AND DEVELOPMENT OF CHLOROPHYLL

COMBINATIONS OF GLASS PLATES	LIMITS OF TRANSMISSION	TRANSMISSION OF AN EQUAL ENERGY SPECTRUM	DEVELOPMENT OF CHLOROPHYLL
	mμ	Per cent.	
G86B	296 – infra red	70.0	Green (control)
G38L	380 – " "	54.0	" as control
G38H	464 – " "	44.5	" " "
G34	528 – " "	33.5	" " "
G24	601 – " "	18.3	" " "
G24 + G55A	670 – " "	6.6	No trace of green*
G24 + G586A	680 – " "	5.7	" " " "
G24 + G585L	690 – " "	2.1	" " " "
G24 + G584J	601 – 680	Less than 0.1	Trace of green
G34 + G584J	528 – 680	1.0	Almost as green as control
G38H + G584J	464 – 680	6.7	" " " " "
G38L + G584J	380 – 680	14.4	Green as control
G34 + G55A	528 – 580	Less than 0.1	No trace of green
G38H + G55A	464 – 580	0.5	Trace of green
G38L + G55A	380 – 580	5.7	Almost as green as control
G38H + G585L	464 – 506	0.2	No trace of green
G38L + G585L	380 – 506	2.6	Almost as green as control
G38L + G586A	380 – 420	0.2	No trace of green
G586A	320 – 420	10.7	Trace of green
G585L	300 – 506	19.3	Almost as green as control
G55A	325 – 580	20.1	" " " " "
G584J	300 – 680	34.8	Green as control

* Faint trace of green in sunflower seedlings in one experiment

different amounts of the green color when compared with plants grown in full daylight. The plants which showed no trace of green were yellow in color similar to etiolated seedlings. These data seem to show two facts:

first, that there is no development of chlorophyll in radiant energy of wave lengths longer than 680 mμ; and second, that all wave lengths of the remaining visible and ultra-violet spectrum are effective if the intensity is high enough. Traces of green color were detected in all combinations where wave lengths shorter than 670 mμ were transmitted except in those where the percentage of total energy transmitted was very low. It must be remembered that the plants were grown in daylight and were not exposed to an equal energy spectrum, so that the transmission percentages are not strictly comparable. It was to get around this difficulty that the series under artificial light was conducted. Here the intensity is constant and the relative spectral distribution and total percentage transmitted can be calculated.

Figure 3 shows the relative spectral distribution of energy under the different combinations of plates used with artificial light. The relative

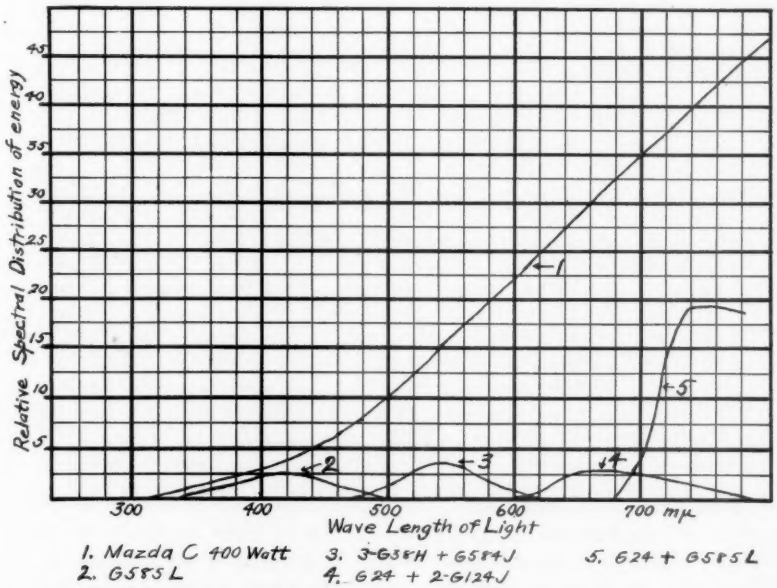

FIG. 3. Relative spectral distribution of energy under different combinations of glasses used.

spectral distribution of energy from the Mazda C 400 watt lamp is also given. The combination 3-G38H + G584J and 624 + 2-G124J were chosen to give values as near as possible to those for G585L. The plants under these plates were all the same distance from the light.

Table II gives the results of an experiment under artificial light. The combinations of plates used, their limits of transmission, and the relative percentage of the total energy from the Mazda C transmitted by each are given. The seeds were germinated and the seedlings grown in the dark to a height of several inches before the light was supplied. The time required for the development of the green color under the different lights is given.

TABLE II

TRANSMISSION DATA AND DEVELOPMENT OF CHLOROPHYLL

Combinations of glass plates	G585L	3–G38H + 584J	G24 + 2–G124J	G24 + G585L
Limits of transmission	320–506 mμ	464–630 mμ	600–800 mμ	680–infra red
Percentage of energy from 400 watt Madza C transmitted	3.3	3.1	4.0	18.0
Time	**Relative development of chlorophyll in the seedlings**			
Feb. 10, '27	Yellow	Yellow	Yellow	Yellow
9:30 A. M.	Yellow	Yellow	Yellow	Yellow
10:15 A. M.	Yellow	Yellow	Yellow	Yellow
11:15 A. M.	Yellow	Yellow	Yellow	Yellow
12 Noon	Yellow	Yellow	Faint trace green	Yellow
1:00 P. M.	Yellow	Faint trace green	Trace green	Yellow
2:00 P. M.	Trace green	Trace green	Green	Yellow
3:00 P. M.	Trace green	Trace green	Green	Yellow
4:00 P. M.	Trace green	Green	Green	Yellow
Feb. 11, '27				
10:00 A. M.	Trace green	Green	Green	Yellow

These data show, as in the other experiments, that even after 24 hours there is not the faintest trace of green in light of wave lengths longer than 680 mμ. The relative intensity of this radiant energy is three times that of the other parts of the spectrum in which chlorophyll development is very pronounced. The data also show that, for approximately equal energy values in the remaining visible and ultra-violet spectrum, there is a difference in their effect on chlorophyll development. This effect seems to be that the red rays are more effective than the green, and the green more than the blue. When the effective energy under the G24 + 2-G124J combination is considered to extend only to 680 mμ, since longer wave lengths

of radiant energy have no effect, the relative transmission percentage becomes 2.5 per cent. instead of 4.0 per cent. as compared with 3.1 per cent. in the green and 3.3 per cent. in the red. The relative effectiveness of the red region is still more apparent.

The fact that there is no development of chlorophyll beyond 680 mμ is very striking because the center of the band of maximum absorption by chlorophyll and the band of greatest efficiency of radiant energy in photosynthesis are at about 675 mμ (SPOEHR). It seems, therefore, that the effectiveness of radiant energy in chlorophyll development increases with the wave length up to about 680 mμ and then ceases abruptly.

Conclusions

1. Wave lengths of radiant energy longer than 680 mμ are not effective in the formation of chlorophyll in seedlings of corn, wheat, oats, barley, beans, sunflowers, and radish.

2. All other regions of the remaining visible and ultra-violet spectrum (to 300 mμ) are effective provided the energy value is sufficient.

3. For approximately equal energy values in these regions, the red rays are more effective than the green, and the green more than the blue.

4. The effectiveness of radiant energy appears to increase with wave length to about 680 mμ and then to end abruptly.

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SIMILARITY BETWEEN PHYSICOCHEMICAL AND BIOLOGICAL REACTIONS*

C. P. Sideris

Students of physiological mycology have long recognized that different microorganisms behave differently both in vigor of growth and in the character of their biochemical reactions when grown on chemically identical substrata. Some of these phenomena have been interpreted rather satisfactorily by certain physicochemical laws, others, however, have either not been explained at all or attributed to the operation of certain vitalistic forces.

Every student of life behavior realizes the difficulties involved in resolving biological phenomena into simple physicochemical reactions. There are occasional cases where one is able to apply certain physicochemical laws. Such a case is presented in the following pages.

There are two proteins with distinct physicochemical properties in the stem of the pineapple plant: Protein-A which is isoelectric at pH 6.4, and protein-B at pH 4.8. Qualitative chemical tests varied in the two proteins as follows:

Protein-A: Biuret (+++), Xanthroproteic (++), Sulphur (−), Molisch (−).
Protein-B: Biuret (+), Xanthroproteic (+), Sulphur (+), Molisch (−).

The separation and treatment of these proteins is discussed in another paper, which will be published elsewhere.

Protein-A has been used for the present study. This protein after a thorough purification by means of dialysis was further diluted with distilled water. This solution was now thoroughly stirred by a mechanical stirrer in order to bring about uniform distribution of the protein particles, and distributed to a number of Erlenmeyer flasks, each one receiving 100 cc. drawn off by means of a pipette. To each one of these flasks different volumes of either 0.1 N. HNO_3 or NaOH were added accordingly to table I.

The table indicates that the isoelectric point of protein-A of the pineapple stem is close to pH 6.43. It, also, shows that this protein may react at pH values below the isoelectric point to form salts, such as protein-

* Published with the permission of the Director as technical paper no. 2 of the Pathological Laboratory of the Experiment Station of the Association of the Hawaiian Pineapple Canners.

TABLE I

BEHAVIOR OF PROTEIN-A WITH ACID AND ALKALI ADDITIONS

SAMPLE NO.	REAGENT ADDED		pH	TURBIDITY	BEHAVIOR WITH SALTS		REMARKS
	HNO_3	NaOH			$K_4Fe(CN)_6$	$AgNO_3$	
	cc.	cc.					
1	4.0		2.63	–	+++	–	
2	3.5		2.67	–	+++	–	
3	3.0		2.70	–	+++	–	
4	2.5		2.88	–	+++	–	
5	2.0		3.00	+	+++	–	
6	1.5		3.13	+	+++	–	
7	1.0		3.33	+	++	–	
8	0.5		4.16	++	++	–	
9	0.25		5.32	+++	+	–	
10	0.125		6.43	++++	–	–	(Isoelectric point)
11	0	0	7.30	++	–	+	
12		0.125	8.65	+	–	++	
13		0.25	9.45	–	–	+++	
14		0.5	9.90	–	–	+++	
15		1.0	10.42	–	–	+++	
16		1.5	10.58	–	–	+++	
17		2.0	11.05	–	–	+++	

ferrocyanate, where the protein takes the place of the cation, and at values above the isoelectric point to form salts, such as silver-proteinate, where the protein takes the place of the anion. HARDY was the first to point out, a number of years ago, that proteins may act as ampholytes. Further investigations and confirmation of this and other properties of proteins came in later years through the researches of PAULI, RONA, MICHAELIS, SØRENSEN, LOEB and others.

The two reactive radicals of a protein that make it amphoteric are the amino (NH_2) and carboxyl (COOH) groups. The former is capable of combining with anions and the latter with cations, to form salts.

Growth behavior of fungi on pineapple protein–A

The different solutions of protein-A as reported in table I were divided into 25-cc. portions and used for the growth of the following organisms:

Fusarium martii,
Verticillium sp.,
Penicillium sp.

Both mycelium and spores were used for the inoculation of the different cultures, the latter being used wherever possible. The cultures were kept at room temperature, that is at about 27° C., and compared with cultures of the same organisms grown on solid and liquid nutrient media. The growth of the different organisms is recorded in table II.

TABLE II

GROWTH OF ORGANISMS ON PROTEIN-A

SAMPLE NO.	pH	GROWTH BEHAVIOR OF		
		Fusarium	*Verticillium*	*Penicillium*
1	2.63	–	+	++
2	2.67	–	+	++
3	2.70	–	++	++
4	2.88	–	++	++
5	3.00	–	++	+++
6	3.13	–	++	+++
7	3.33	–	+++	+++
8	4.16	–	++	++
9	5.32	–	++	++
10	6.43	–	–	–
11	7.30	+	–	+
12	8.65	++	–	++
13	9.45	+++	–	++
14	9.90	+++	–	++
15	10.42	++	–	++
16	10.58	++	–	++
17	11.05	+	–	+

Discussion

Protein as such is not available for the use of a fungus. We know that organisms release enzymes to decompose and make available not readily available substances. Very little is known of the operative mechanism of enzymes, because there is no satisfactory evidence of their chemical constitution. Certain investigators have stated at different times that enzymes that decompose proteins are proteins and those that decompose carbohydrates are carbohydrates. Just how true this statement is the writer is not in a position to say. If we take it for granted, however, that the case may be such and attempt to interpret the results obtained with the different organisms accordingly, we have the following condition.

Assuming, *a priori,* that the enzyme released by each of the three different organisms and causing the breaking down of protein-A is of protein composition, we may be justified then in attributing its chemical reactivity

to either one of its two active component radicals, namely, NH_2 or COOH. With the above assumption in mind one may easily visualize the reactions taking place between the enzymes of *Fusarium* and *Verticillium*, on the one hand, and the pineapple protein, on the other, but it is considerably more difficult to understand the operative mechanism of *Penicillium*.

In order to make some of the points more clear let us consider the types of chemical reactions that are possible in each case.

It is possible that the condition of the protein at the isoelectric point, and above or below this point, is as follows:

Isoelectric point, HOOC—R—NH_2

Below isoelectric point, HOOC—R—$NH_2 + H^+ \rightarrow$ (HOOC—R—NH_3)$^+$

Above isoelectric point, HOOC—R—$NH_2 + OH^- \rightarrow$ (OOC—R—NH_2)$^- + H_2O$

The very same types of conditions may be ascribed to the enzymes of the different organisms according to our assumption that they are of protein composition. As the isoelectric point of the different enzymes is not known it is difficult accurately to know their reactivity at different pH values. The results already obtained indicate that the enzyme of *Fusarium* operated only at pH values above the isoelectric point of the pineapple protein and that of *Verticillium* at values below the isoelectric point. This sort of behavior reveals the presence of two enzymes with opposite electrical charges, that of *Fusarium* carrying a positive and that of *Verticillium* a negative charge.

One wonders whether the enzymes of *Fusarium* and *Verticillium* are of proteinaceous composition or not. They do not seem to be amphoteric but are charged either positively or negatively through a wide range of H-ion concentrations, judging from their reactivity with the pineapple protein. Exactly what conditions prevail with the enzyme of *Penicillium*, which shows amphoteric behavior, is difficult to explain. From the evidence presented so far, it is shown that the latter enzyme must be affected somewhat differently than the pineapple protein by hydrogen ions, and it assumes an electric charge opposite to that of the protein. In accordance with our former assumption it is possible to have the following conditions with the different enzymes:

For *Fusarium:*

At the isoelectric point

(HOOC–protein–NH_2) + (HOOC–enzyme–NH_3)$^+$ = no reaction.

Above the isoelectric point

(OOC–protein–NH_2)$^-$ + (HOOC–enzyme–NH_3)$^+$ ⟶ HOOC–protein–NH_2 + HOOC–enzyme–NH_2 = reaction.

Below the isoelectric point

$(HOOC–protein–NH_3)^+ + (HOOC–enzyme–NH_3)^+ =$ no reaction.

For *Verticillium:*

At the isoelectric point

$HOOC–protein–NH_2 + (OOC–enzyme–NH_2)^- =$ no reaction.

Above the isoelectric point

$(OOC–protein–NH_2)^- + (OOC–enzyme–NH_2)^- =$ no reaction.

Below the isoelectric point

$(HOOC–protein–NH_3)^+ + (OOC–enzyme–NH_2)^- \longrightarrow HOOC–protein–NH_2 + HOOC–enzyme–NH_2 =$ reaction.

It is questionable in the case of *Penicillium* whether there is only a single enzyme operating throughout such a wide range, namely, pH 2.0 to 11.00. There might be two enzymes, one carrying a negative electric charge and operating below the isoelectric point of the protein and the other with a positive electric charge and operating above the isoelectric point. Whether similar conditions are to be found with other proteins and fungi remains to be seen and further studies are in progress.

Summary

An attempt is made in this study to compare biological with physicochemical reactions and to interpret the former in terms of the latter. To just what extent this is feasible remains to be proved by further experimentation.

It has been found that *Fusarium martii, Verticillium* sp. and *Penicillium* sp. do not grow in isoelectric pineapple stem protein-A. *Fusarium* may grow in solutions of this protein having a pH value above that of the isoelectric point of the protein. *Verticillium* behaves exactly reverse, that is, it grows only in solutions of the protein having pH values below that of the isoelectric point. *Penicillium* sp. was found to grow in solutions of the protein having a pH value either above or below the isoelectric point.

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PHOTOSYNTHESIS IN ABSENCE OF OXYGEN*

E. NEWTON HARVEY

BEIJERINCK (1) was the first to use luminous bacteria as detectors of oxygen in the study of photosynthesis. He found that extracts of destroyed cells containing chloroplasts, mixed with luminous bacteria whose luminescence had disappeared because of lack of oxygen, would, when illuminated, produce photosynthetic oxygen that was detected by the luminescence of the bacteria.

MOLISCH (5, 6) later found that leaves carefully dried, powdered, and suspended in emulsions of luminous bacteria could also photosynthesize when illuminated.

The simplicity and sensitivity of this method of study make it especially valuable for exploratory studies on photosynthesis under different conditions and especially for studies on photosynthesis in absence of oxygen, since the luminous bacteria themselves may act as the means of absorbing oxygen.

Statements in the literature as to the necessity of oxygen for photosynthesis are somewhat conflicting, but the general conclusion seems to be that oxygen is necessary (7, 8), different plants differing in their resistance to lack of oxygen. WILLSTÄTTER and STOLL (10) found *Pelargonium* to be quite sensitive, while *Cyclamen* was very resistant to lack of oxygen.

Despite the statements as to the necessity of oxygen for photosynthesis there is no theoretical reason why the first step in photosynthesis, the splitting of CO_2, which is the step detected by luminous bacteria, should fail in absence of oxygen. It would seem that further experiments in which it is certain that the oxygen is completely removed are desirable.

In many experiments where gases are passed over cells to remove the oxygen, the gases may contain traces of oxygen which are easily detected by the luminescence of luminous bacteria. HARVEY and MORRISON (3) have found that light can just be detected in an emulsion of luminous bacteria 3 cm. thick when in equilibrium with 0.0053 mm. O_2 or 0.0007 volume per cent. of oxygen. This will give some idea of the sensitivity of luminous bacteria as oxygen detectors. However, an emulsion of luminous bacteria becomes completely dark if undisturbed, and can reduce many dyes whose oxidation-reduction potential is well known. A knowledge of the potential of the dye allows us to calculate the pressure of oxygen with which equimolecular quantities of reduced and oxidized dye would be in equilibrium.

* From the Marine Biological Laboratory, Woods Hole, Massachusetts.

The particular strain and concentration of bacteria used in these experiments reduced methylene blue in 3–4 minutes and indigo disulphonate in about an hour. Indigo monosulphonate was not reduced.

The potential of an equimolecular mixture of reduced and oxidized methylene blue is about +0.02 volts at pH = 7 which corresponds to an oxygen pressure of about 10^{-53} atmospheres, a figure that has no physical significance. We may at least say that the last molecule of oxygen is removed by luminous bacteria and that they produce perfect anaerobic conditions.

As luminous bacteria are marine forms and live well when emulsified in sea water, I have added various kinds of marine algae to tubes containing emulsions of luminous bacteria which had become dark through using of oxygen. After remaining in the oxygen-free sea water for a given time the algae were illuminated with a 60 watt straight filament Mazda lamp at a distance of 12 cm. for a few seconds.

Not only do the bacteria use oxygen but they also produce CO_2, changing the pH of the sea water from 8 to 6.9–7 in the course of an hour. A pH of 7 corresponds to 4 mm. CO_2, according to HENDERSON'S and COHN'S (**4**) sea water pH–CO_2 pressure curve. On blowing air through the sea water containing the bacteria its pH returns to about 8 showing that non-volatile acids are not produced in large amount. The bacteria may then be regarded as ideal means of removing oxygen since they at the same time add the CO_2 necessary for photosynthesis.

The actual results are given in table I. The algae were recently collected, and kindly identified for me by Dr. W. R. TAYLOR. The number of + signs indicates roughly the intensity of the luminescence, and the – signs mean no luminescence.

It will be observed that nearly all the algae readily form oxygen under the conditions of the experiment. Since *Grinellia* gave no luminescence I concluded that the specimen was either dead or in poor condition. This experiment will, therefore, serve as a control to show that luminous bacteria themselves produce no oxygen or luminescence when illuminated. It should be mentioned that they do luminesce when suspended in sea water after exposure in a quartz tube to light from a quartz Hg-vapor lamp. One might suppose that this experiment indicated a splitting of the CO_2 of sea water by ultra-violet light, but it is perhaps better interpreted as a production by the ultra-violet light, of H_2O_2, which then decomposes with liberation of oxygen. TIAN (**9**) found H_2O_2 to be formed in absence of oxygen when sea water was exposed to ultra-violet. The convenience of luminous bacteria as oxygen detectors suggests their value in experiments on photosynthesis *in vitro* upon which I am at present engaged.

TABLE I

PHOTOSYNTHESIS OF MARINE ALGAE

PLANT	OXYGEN PRODUCTION ON ILLUMINATION AFTER OXYGEN-FREE CONDITIONS FOR				
	1 minute		15 minutes	30 minutes more	
	Illuminated		Illuminated	Illuminated	
	3 sec.	30 sec.	3 sec.	3 sec.	30 sec.
Green algae					
Cladophora gracilis	+	+		+	
Bryopsis plumosa	+	+		+	
Enteromorpha intestinalis	+	++		+	
Ulva lactuca rigida	+	+	+	+	
Chaetomorpha linum	++	++	++	++	
Red algae					
Polysiphonia variegata	+	–		+	–
Polysiphonia violacea	+	++	+	+	
Polysiphonia nigrescens	+			+ faint	
Chondrus crispus	+	+	+	+	
Lomentaria uncinata	+	+	+	+	
Corallina officinalis	+	+	+	+	
Grinellia americana	–	–	–	–	–
Brown algae					
Fucus vesiculosus	++	+++	++	++	
Ascophyllum nodosum	++	+++	++	++	
Ectocarpus siliculosum	+ faint	– ?	+ faint	+	
Eel grass (*Zostera marina*)	++	++	+ faint	–	–

It has frequently been noticed that with some algae a short exposure to light caused oxygen production which stopped on longer illumination, possibly due to the fact that the light was too intense. This observation requires further investigation to make certain of the explanation.

Another point of interest is that oxygen is produced as quickly as the lamp can be turned on and off and I can confirm BEIJERINCK'S statement that striking a match is sufficient to cause photosynthesis. The very rapid appearance of oxygen outside the cells of the plant, where it is detected by the bacteria is only further evidence of the rapidity with which oxygen passes the plasma and cellulose membrane of the cells (**2**). Oxygen is a very freely penetrating substance.

Fucus vesiculosus gave the best light of all. It was, therefore, placed in the dark with an emulsion of luminous bacteria through which a slow current of hydrogen was passed. The hydrogen was conducted over red

hot platinized asbestos, thus rendering it absolutely free of oxygen, and led to the bacteria in a lead tube sealed to glass with DE KHOTINSKY'S cement. If luminous bacteria are to be kept in good condition in absence of oxygen, the CO_2 must be largely driven off and this was accomplished by the hydrogen. After 2 hours the *Fucus* was illuminated and found to photosynthesize since the bacteria glowed. After 2 more hours in oxygen-free darkness the *Fucus* was again illuminated and found to photosynthesize again. Since the luminous bacteria reduce methylene blue in a few minutes and indigo-carmine in about one hour we can state that *Fucus* (and many other algae) can produce oxygen from CO_2 under complete anaerobic conditions, even after 2 hours anaerobiosis.

The only form which showed any stopping of photosynthesis in absence of oxygen was the eel-grass. Even this plant could photosynthesize in absence of oxygen after 15 minutes, but not after 30 minutes more. I am, therefore, led to the conclusion that the first step in photosynthesis in marine algae and in eel-grass does not require oxygen. Whether sugar or starch is formed in absence of oxygen I cannot say.

Summary

Fourteen species of marine algae, including members of the green, red and brown groups, are able to produce oxygen from carbon dioxide when illuminated in complete absence of oxygen.

The oxygen appears within a second after illumination, showing the ready permeability of the plasma and cellulose membranes to this gas.

Luminous bacteria are recommended as a test for oxygen under all conditions where they will survive.

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A STUDY OF THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF *AGARICUS CAMPESTRIS* *

DONALD FREAR, J. F. STYER AND D. E. HALEY

(WITH ONE FIGURE)

Introduction

It has long been known that *Agaricus campestris*, the common mushroom, grows well on horse manure which has been partially decomposed. In order for fruition to occur, however, the manure must be covered with a thin layer of soil shortly after a good mycelial development has been obtained in the manure. Under these conditions mushrooms develop and mature over a period of several months. At the end of that time, however, the production ceases and before the medium has been depleted of nutrients. The cause of this may be the exhaustion of some food or food material, a change in the physical substratum, the formation of a toxin, or a change in the reaction of the medium. Then again it may be due to a combination of these and possibly other factors. In order to make a study of any single factor affecting the growth of mushrooms, it is highly desirable, if not imperative, to grow mushrooms in pure cultures.

Very little investigational work has been done on the growing of mushrooms in artificial media. Several French investigators, notably BOYER (**1**), have grown the mycelium on agar containing plant decoctions. BOYER found that the best growth was obtained where carrots, finely ground, were used as the basis of the culture. DUGGAR (**2**), and FERGUSON (**3**) also successfully grew mushrooms on synthetic cultures of known composition, although they did not attempt to carry their experiments any farther.

The object of this experiment was to determine, if possible, the optimum H-ion concentration for the growth of the mycelium of *Agaricus campestris* and to determine the effect of the organism upon the reaction of the nutrient medium.

Experimental

The medium used for the growth of the mycelium of the mushroom in question was that used by STYER (**4**), who, in cooperation with Dr. TRUE, of the University of Pennsylvania, found that a fair growth could be obtained with the following mixture:

* Approved by the Director of the Experiment Station as Technical Paper no. 440.

Black filter paper (Whatman's no. 29)	10.0 gm.
Casein	0.6 gm.
20 cc. of the following solution:	
$MgSO_4 \cdot 7\ H_2O$	0.02 M.
KH_2PO_4	0.04 M.
K_2SO_4	0.01 M.
$FeSO_4 \cdot 7\ H_2O$	Trace
$CaCl_2$	Trace

The differences in hydrogen-ion concentration were obtained by adding varying amounts of acid (H_2SO_4) and base (KOH). One set of cultures (P) was made up using 10 grams of the black filter paper plus 20 cc. of distilled water.

The culture medium was placed in 300 cc. Pyrex Erlenmeyer flasks and the flasks plugged with cotton to avoid contamination, after which they were sterilized in a steam autoclave at 15 pounds pressure and 120° C. for 30 minutes. The flasks were then inoculated by introducing a small piece of mycelium from a pure culture growing in manure. The check flasks were treated in the same manner as the others, except that they were not inoculated.

After inoculation the flasks were removed to a light-proof incubator, which was kept at 27° C. by means of an electric thermostat. The humidity was preserved by means of pans of moist sphagnum moss, in which the flasks were placed. Observations were made and the growth recorded each week in terms of the diameter of the colony in centimeters. The flasks were uniformly 8 cm. in diameter at the base.

The media were extracted with 50 cc. of distilled water, and the H-ion

TABLE I

COMPARISON OF THE INITIAL pH OF THE MEDIA WITH THE FINAL pH

CULTURE	pH	
	INITIAL	AFTER 4 WEEKS
C	3.4	3.5
D	3.8	4.6
E	4.1	5.3
F	4.5	4.7
G	5.1	5.0
H	5.5	5.4
I	6.0	4.0
J	6.3	4.3
K	6.7	4.7
L	7.0	5.6
M	8.0	7.6
P	7.0	5.0

concentration of the resulting solutions were obtained by means of the potentiometer, using Bailey electrodes.

The data given in tables I and II were the averages obtained from three cultures grown under exactly the same conditions. The curve of growth with change in pH is shown in fig. 1.

Summary and conclusions

From the foregoing figures it may be seen that the best growth was obtained in the cultures which were nearest to a pH of 6.0, although the organism was tolerant to all conditions which were imposed upon it. The cultures in which the growth was greatest changed the reaction of the media

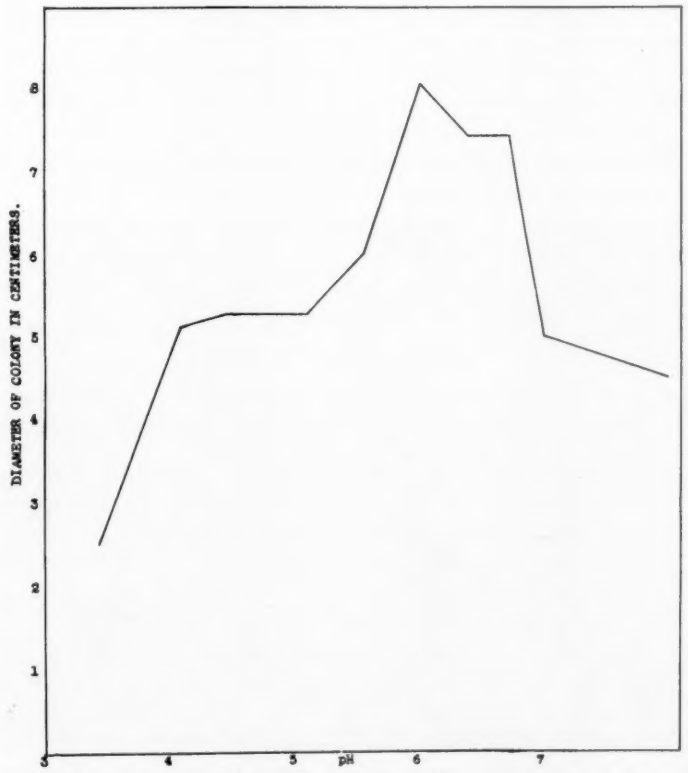

FIG. 1. Growth of *A. campestris* in relation to the H-ion concentration of the medium.

TABLE II

GROWTH OF *Agaricus campestris* AS AFFECTED BY THE H-ION CONCENTRATION OF THE NUTRIENT MEDIUM

CULTURE	pH	GROWTH (DIAMETER IN CM.)			
		1 week	2 weeks	3 weeks	4 weeks
C	3.4	0.4	0.8	2.0	2.5
D	3.8	1.0	3.0	4.2	4.2
E	4.1	1.0	3.0	4.2	5.1
F	4.5	1.0	3.8	4.7	5.2
G	5.1	1.2	4.4	5.0	5.2
H	5.5	1.4	4.6	5.0	6.0
I	6.0	2.0	5.0	7.0	8.0
J	6.3	1.9	5.3	7.0	7.4
K	6.7	1.4	5.6	7.0	7.4
L	7.0	0.5	3.6	5.0	5.0
M	8.0	0.4	2.0	4.0	4.5
P	7.0	0.5	1.0	2.0	2.0

to a marked extent, as evidenced by the marked difference in the initial and final pH in cultures I, J, K, and L.

The authors wish to thank Dr. R. H. TRUE, of the University of Pennsylvania, who suggested this work.

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EFFECT OF HIGH TEMPERATURE ON CHARACTER OF GROWTH OF CABBAGE

(WITH ONE FIGURE)

In a study of the rest period of cabbage, the writer found that full grown plants, which were removed to a greenhouse early in the fall, would not go to seed under relatively high temperatures. The plant shown in the figure was, with others, removed from the field October 8, 1925, and brought direct to the warm greenhouse in which the temperature averaged 70° F. This lot of plants was used as checks for comparison with others which were given a rest period. A similar lot was placed, for the same purpose, in the cool house in which the average temperature was 60°. None of the controls in the warm house developed seed-stalks but continued vegetative growth, and by March 7, 1926, all the plants had developed compact

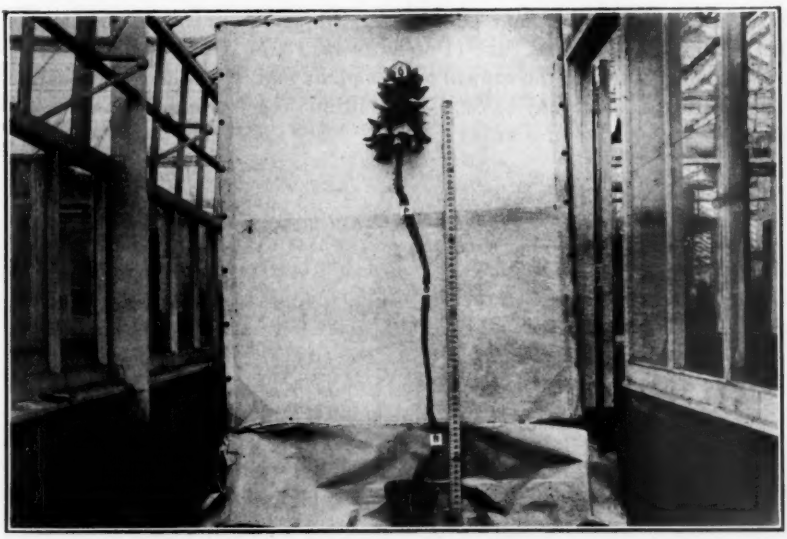

FIG. 1. N—Mature head, Oct. 8, 1925. O—Mature head, March 7, 1926. P—Mature head, Sept. 20, 1926. Q—Mature head, March 1, 1927, and a fifth head matured by September 15, 1927.

heads. The control plants in the cool house developed seed-stalks and flowers in 154 days. Similar lots of plants which had been allowed a rest period from October 8 to December 12 at a temperature ranging from 35°–45° F. were removed to the warm and cool greenhouses on December 12th. These plants, having had the above rest period, developed seed-stalks and mature flowers in the warm house in 39 days, while those in the cool house developed mature flowers in 68 days. Five of the heads were allowed to remain in the warm house for further observation.

About June 10th the heads cracked, vegetative growth continued, and by September 20th a third head had formed. By March 1, 1927, when the photograph was made, the plant illustrated had developed a fourth head. The fifth head was fully developed by September 15, 1927. The plant has been shifted to a pot containing new soil twice each year. At the present time it is growing in a wooden box 16 x 18 inches. As can be seen from the photograph, it is over six feet tall and at the present date it is at least eighteen inches taller than when the photograph was made.

The vegetative growth of cabbage plants in the warm house seems to agree well with the work on the beet (*Beta vulgaris* L.), by KLEBS, who states that it usually behaves as a biennial but that the plant may not be able to flower the second season if kept in the warm greenhouse during the winter. This cabbage plant is now over two years old, has produced five heads and at the present time (December 15, 1927) is growing vigorously. It seems evident from the growth of this plant that, with the control of the temperature, a plant may be caused to continue vegetative growth.—JULIAN C. MILLER, *Cornell University*.

NOTES

The Nashville Meeting.—The meeting held at Nashville last December demonstrated the growing strength and vitality of the American Society of Plant Physiologists. The membership was well represented in the attendance, and the programs were of good quality throughout the sessions. Much interest centered in the annual dinner for all plant physiologists, the evening of Dec. 28. Seventy-five members and friends of the Society were in attendance, and the dinner was made the occasion of a celebration of the 250th anniversary of the birth of STEPHEN HALES, and the 200th anniversary of the publication of his most famous contribution to botanical science, Vegetable Staticks. The interesting story of his life and works was given by the president as a feature of the after-dinner program. Following this sketch of HALES's life, was the official announcement of the establishment of the STEPHEN HALES Prize Fund, by BURTON E. LIVINGSTON. The main facts concerning this fund are recorded in another paragraph in this number of Plant Physiology.

The dinner was also made the time and place of the announcement of the second award of the CHARLES REID BARNES honorary life membership in the Society. This announcement was made by Professor FRANK M. ANDREWS, chairman of the award committee. These events made the occasion memorable, and those who were fortunate enough to be present have carried away the happy memories and inspiration of a very enjoyable meeting.

The Stephen Hales Prize Fund.—The effort to create a prize fund in honor of STEPHEN HALES met with a generous response on the part of the members and friends of the American Society of Plant Physiologists. The amount which had been contributed to the fund at the time of the meeting was slightly in excess of $1000. Gifts since have brought the fund to $1031.75, which is a very fine beginning. In order that an award might be made at the New York meeting in 1928, one of the most generous donors to the fund stipulated that $100 should be set aside, and kept uninvested for the 1928 award. This leaves $931.75 in the fund for investment. It has been decided to keep the fund open, so that additional gifts may be made. A number of individuals found it impossible to assist at the time the opportunity for contributions was given. Any one who would like to have a share in the building of this fund, has the privilege of making additions to it at any time by sending a check for any amount he desires to

give, to Dr. S. V. EATON, the secretary-treasurer, Dept. of Botany, University of Chicago. As the fund increases it may be possible to award a STEPHEN HALES Prize every year, or to increase the amount of the award if it remains a biennial occurrence.

The task of devising methods of handling the funds and making the awards is in charge of a committee of which Dr. JAMES G. PEIRCE of Stanford University is chairman. The other members on the committee are FRANCIS E. LLOYD, E. J. KRAUS, J. B. OVERTON, and B. E. LIVINGSTON. The establishment of the STEPHEN HALES Prize Fund on such a substantial basis reflects the earnestness, vigor, and enterprise of this organization.

The Life Membership Award.—The second award of the CHARLES REID BARNES honorary life membership in the American Society of Plant Physiologists was made to Professor FRANCIS E. LLOYD, MacDonald Professor of Botany in McGill University. An account of the award appears in Science for March 19th. Professor LLOYD has been a very able investigator in the field of plant physiology, and has made many worthwhile contributions to our knowledge in this field. During recent years his studies of the fluorescence of plant pigments, the physiology of conjugation in *Spirogyra*, and the habits of *Vampyrella* have attracted wide attention. His moving pictures of gametic fusion, contractile vacuole activity, and feeding habits of *Vampyrella* have been entertaining and instructive features of the annual meetings of the Society for several years. The American Society of Plant Physiologists, in this award, has expressed its sense of appreciation of his valuable services. Professor LLOYD forms the second member in the living link memorial which was established in honor of BARNES at the Kansas City meeting in 1925. The two members thus honored are:

BURTON E. LIVINGSTON, Johns Hopkins University,
FRANCIS E. LLOYD, McGill University.

Sixth National Colloid Symposium.—The sixth national colloid symposium will be held at Toronto, Canada, June 14–16, 1928. The guest of honor this year will be Professor WILLIAM B. HARDY, of the University of Cambridge. These meetings are always valuable, and any one who desires to attend is welcome. A small registration fee is usually charged, to cover the cost of certain of the social features. Plant physiologists have in the past found these meetings very much worthwhile.

Agronomy Meeting.—The Corn Belt Section of the American Society of Agronomy will hold the summer meeting at Columbus, and Wooster, Ohio. The meeting will begin at Columbus on June 21, 1928. In the late afternoon the scene will be shifted to the Experiment Station. Those in

attendance will be taken by auto to Wooster, where the meetings will continue on June 22 and 23. Last year the meeting was to have been held at Columbus, but the meeting had to be postponed because of other meetings.

Errata.—The attention of members and subscribers to Plant Physiology is called to the list of *errata* published at the close of the table of contents in the October, 1927 number. The editor regrets the occurrence of these errors, particularly those in connection with the citation of literature in one of the reports of the committee on methods of analysis. It is suggested that readers enter these corrections at the places where the errors occur. All members are invited to assist the editor in detecting and correcting any errors which are found in text or tables of papers published in the journal.

Program Committee.—The program committee for the New York meeting in December, 1928, consists of the following members: Professor D. R. HOAGLAND, University of California, chairman; Professor G. N. HOFFER, Purdue University, and Professor WILLIAM SEIFRIZ, University of Pennsylvania. The secretary-treasurer, Dr. SCOTT V. EATON, the University of Chicago, is *ex-officio* a member of the committee. Members of the society can cooperate with the committee by offering their best contributions for the program of the New York meeting. This will be the fifth annual meeting of the Society. May we not make it an outstanding meeting?

Investigations on Chlorophyll.—This splendid contribution by WILLSTÄTTER and STOLL has been given English translation by Dr. FRANK M. SCHERTZ and Dr. ALBERT R. MERZ, both of the Department of Agriculture. The book should appeal to many students, as the original German is not always easily read. The book has been printed on excellent paper by the Science Press Printing Co., Lancaster, Pennsylvania, and is nicely bound in cloth. As the book has been published by the senior translator on his own capital, members of the Society can show their appreciation by ordering a copy of the work, and placing copies in the libraries of our respective institutions. As it can be purchased only from the senior translator, his address is given in full. The price is $4.50, and can be had from Dr. FRANK M. SCHERTZ, 1305 Farragut St., N. W., Washington, D. C.

Physical Chemistry and Biophysics.—This book has been prepared for students of biology and the medical sciences, by Dr. MATTHEW STEEL, Professor of Biological Chemistry in the Long Island College Hospital, Brooklyn, N. Y. There are thirteen chapters, the first of which is a brief introduction. The chapter headings include the following: The nature

and structure of matter; general properties of matter; energy transformations in living matter; general nature of solutions; water, the greatest solvent; diffusion and osmotic pressure; the nature and behavior of electrolytes in solution; chemical equilibrium and the law of mass action; measurement of hydrogen-ion concentration; the colloidal state of matter; catalysis and velocity of chemical reactions; and dynamical physical chemistry of the cell.

The book is well written, and will be a very valuable aid to those who need the fundamental knowledge of the physical and chemical dynamics of life. It is more than usually helpful, and should have a warm reception among plant physiologists. The price of the book is $4.00, and is published by John Wiley and Sons, New York.

Biochemical Laboratory Methods.—At the time of his death, the late Professor CLARENCE A. MORROW, of the University of Minnesota, left the unfinished manuscript of a manual of Biochemical Laboratory Methods. This work has been brought to completion with the aid of Drs. R. A. GORTNER and T. A. PASCOE, and has been published for Mrs. MORROW by John Wiley and Sons. The experiments are arranged in nine chapters: The colloidal state; physical chemical constants of plant saps; hydrogen-ion concentration and buffer action; proteins; carbohydrates; glucosides; fats and allied substances; enzymes; and plant pigments. There are 233 experiments outlined, and any student who masters the methods given in this book will be a well trained biochemist. The directions given are clear and detailed. This is another book which can be recommended to the plant physiologist for his private library, and is not too expensive. The price is $3.75, and may be ordered from John Wiley and Sons, New York.